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JOURNAL OF ECONOMIC ENTOMOLOGY

ENTOMOLOGICAL SOCIETY OF AMERICA

Vol. 52 August, 1959 No. 4

Genetic Control of Size in Queen Honey Bees[1]

ROGER HOOPINGARNER and C. L. FARRAR, *Department of Entomology, University of Wisconsin Agricultural Experiment Station, Madison*[2]

ABSTRACT

Four inbred lines of honey bees (*Apis mellifera* L.) were analyzed for weight differences. Two lines were compared with their F_1 hybrids. A highly significant difference was shown between the inbred lines and between the inbred lines and their F_1 hybrids; this indicates heterosis for body weight in the lines used.

A proportional relationship of body weight to number of ovarian tubules is shown; the correlation between these variables is highly significant.

The phenotypic differences between queen honey bees (*Apis mellifera* L.) are often very striking and can be of economic importance to the colony and to the beekeeper. These variations may be caused by either environmental or genetic factors. In a study of the environmental factors affecting queen bees, genetic control came under consideration as a means of reducing variability of the test stock.

Ruttner & Mackensen (1952) state that tongue length, body size, and some other anatomical characteristics of adult bees are heritable. Roberts (1956) found significant differences in the weight of worker bees between various inbred lines.

The increase in size of the queen appears to occur mostly in the abdomen. The number of ovarian tubules, as part of the abdomen, may increase proportionally. Eckert (1934) found no significant differences in the number of ovarian tubules of larvae grafted at various ages from 12 to 72 hours, and no high correlation between various body measurements and the number of ovarian tubules in the queen. Weaver (1957) has shown a variable correlation between the size or weight of the queen and the number of ovarian tubules. Therefore, body weight of queens was analyzed in some inbred lines of honey bees.

MATERIALS AND METHODS.—The four inbred lines (3, 6, 9, and Z) used in this study were from the stocks of the North Central States Bee Culture Laboratory at Madison, Wisconsin. In addition the reciprocal F_1 hybrids between 3 and 6 were tested. All matings for maintenance of the lines were by artificial insemination. The queens were raised in queen rearing colonies with environmental differences at a minimum.

Four days before grafting, empty combs were put into the small colonies (shallow 4-frame nuclei), each containing an inbred queen being tested. The combs were removed 18 to 20 hours afterward and put into a strong colony separated from the queen by an excluder. The larvae were grafted into a bed of royal jelly provided by preliminary grafted larvae using the "swarm box" technique.

On the 11th day after the eggs were laid the queen cells were removed from the colonies and the pupae were weighed. The 11th day was chosen, as this period showed the least variation of all points along the entire growth curve (Hoopingarner & Farrar, unpublished). Six pupae were weighed from the center of each cell bar. The analyses of variance showed that the sampling error was equal to or only slightly greater than the experimental error, indicating this is a reliable sample size.

In comparison of two lines, both were grown together in the same colony and in adjacent bars, with positions randomized to minimize any confounding of line differences with intra hive environmental differences. In the analysis of variance the variation between bars within a colony was used as the error term.

RESULTS.—The experiments were grouped into sets of two, as this was the most convenient way to time the age of the eggs and to graft the larvae. One inbred line (line 6) was used in each test as a period or seasonal comparison. The results are summarized in table 1.

To determine if weight increase was beneficial, a comparison was made between the number of ovarian tubules and body weight. Pupae of the various weight classes were fixed in KAAD (kerosene-alcohol-aceticacid-dioxan) imbedded in paraffin (56°–58° C.), and sections cut from the abdomens. The sections were mounted, stained, and the ovarian tubules counted. The results are shown in figure 1.

DISCUSSION.—This experiment was undertaken to see if the apparent visual differences in size between the inbred lines were real. The differences in weight between lines in this experiment far exceeded the normal level of significance. It was necessary to assess these weight differences in reference to better queens. The counting of ovarian tubules was then essential even if more tubules in heavier queens had been indicated by Weaver's (1957) data. The regression of ovarian tubules on weight is probably curvilinear with sharp breaks between the dimorphic castes. In the range of normal queens in this experiment a straight line relationship indicates a proportional increase of tubules with increase in body weight.

The question of whether more ovarian tubules increase egg laying is beyond the scope of this experiment; how-

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[2] In cooperation with the Entomology Research Division, Agric. Res. Serv., U.S.D.A.

Table 1.—Comparisons between the inbred lines of honey bee queens.

EXPERIMENT	LINE	INBREEDING COEFFICIENT	N	MEAN WEIGHT (MG.)	DIFFERENCES	ERROR DEGREES OF FREEDOM	T-VALUES	P
1	6	0.791	48	278.07	5.02	11	4.84	<0.001
	Z	.889	48	273.05				
2	6	.791	36	274.88	12.57	8	5.36	<0.001
	9	.441	36	262.31				
3	6	.791	24	275.61	5.58	5	2.13	>0.1
	3	.724	24	270.03				
4	6	.791	48	276.38	12.06	11	5.27	<0.001
	3×6	—	48	288.44				
5	6	.791	36	278.82	14.00	8	8.12	<0.001
	6×3	—	36	292.82				

ever, the number of ovarian tubules should have a direct positive correlation with the number of eggs the queen is capable of laying in a given time. This should be true when the physiological and environmental factors are equal. Large inbred lines would then be advantageous, if the genes for body weight and/or ovarian tubules are additive in inheritance.

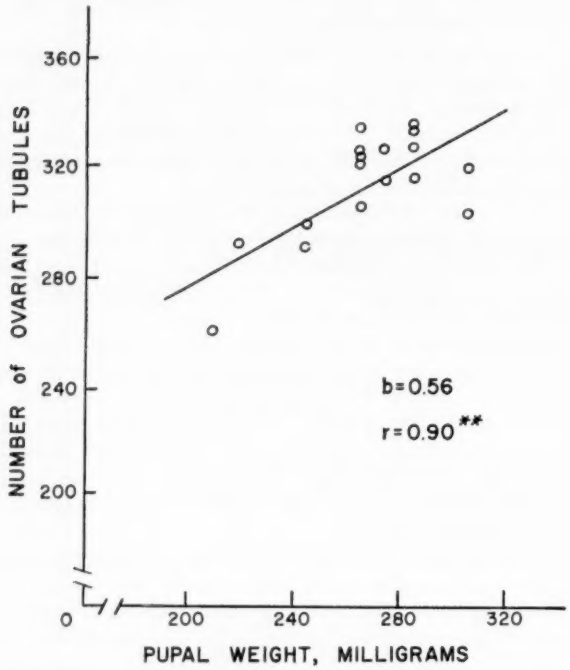

FIG. 1.—Total number of ovarian tubules, in histological cross section, for pupal weights.

It was not known prior to these tests whether the hybrids of experiments 4 and 5 would show heterosis even though Cale (1956) found heterotic effects in oviposition and honey yield. Schwartz (1953) had found slight heterotic effects in some anatomical measurements but not in the dry weight of worker bees. It is apparent from table 1 that heterosis for body weight of queens is very highly significant ($P<0.001$).

Selection of inbred lines of honey bees can, therefore, be made on mean weights of the queens. If dominant and/or epistatic gene factors are significant in the inheritance of body weight or ovarian tubules, screening could be done on the hybrid combinations.

REFERENCES CITED

Cale, G. H., Jr. 1956. Heterosis in the honey bee (*Apis mellifera* L.). Genetics **41**: 292–303.

Eckert, J. E. 1934. Studies in the number of ovarioles in queen honeybees in relation to body size. Jour. Econ. Ent. **27**: 629–734.

Roberts, W. C. 1956. Hybrid vigor in honeybees. 16th Internatl. Beekeeping Congr. Prelim. Sci. Meeting. Vienna Congress. Abst. Bee World **37**: 203, Oct.

Ruttner, F., and O. Mackensen. 1952. The genetics of the honey bee. Bee World **33**: 53–62, 71–79.

Schwartz, E. L. 1953. Heterosis in the honey bee. Ph.D. Thesis, University of Wisconsin.

Weaver, N. 1957. Effects of larval age on dimorphic differentiation of the female honey bee. Ann. Ent. Soc. America **50**: 283–94.

The Effects of Field Applications of Some of the Newer Pesticides on Honey Bees[1]

FRANK R. SHAW, *Massachusetts Agricultural Experiment Station, Amherst*

ABSTRACT

From these experiments residues of 0.25 lb. actual dieldrin per 100 gallons of spray may be highly toxic to honey bees (*Apis mellifera* L.) for periods at least up to 96 hours after application. Sevin® (1-naphthyl N-methylcarbamate) wettable powder, 50%, or a 36% mull at dosages of 1 lb. actual per 100 gallons was highly toxic as a contact poison. Residues of 1 lb. actual Sevin were highly toxic for a period of 24 hours after application. Their toxicity diminished after a period of 96 hours. The toxicity of Am. Cyanamid No. 5223 (Cyprex® = dodecyl guanidine acetate) at a dosage of 1 lb. per 100 gallons of water was not significantly different from that of the control bees.

With the increasing number of pesticides being developed to control insects and other pests, there is a very real need to determine the effects of such materials on honey bees (*Apis mellifera* L.) under field conditions. The present investigation was concerned with the effects of Sevin® (1-Naphthyl N-methylcarbamate) dieldrin, and Am. Cyanamid No. 5223 (Cyprex® = dodecylguanidine acetate).

REVIEW OF LITERATURE.—There is little or no published information on the effects of these pesticides on bees under field conditions, and only a limited amount of data under laboratory conditions.

In the laboratory, Anderson & Tuft, 1952, exposed caged bees to various pesticide dusts using a dosage of 0.25 gram in a bell-jar-type duster. They report that a 1% dieldrin dust produced 100% mortality of honey bees within 6 hours, whereas a 5% DDT dust required 18 hours to produce the same results. Anderson & Atkins (1958) reported that a dust containing 2.5% of Sevin at a 200-mg. dosage in a bell jar type of duster produced 72% mortality within 24 hours, whereas the same level of a 5% DDT dust killed only 52%.

Anderson & Tuft (1952) reached the following conclusions: "Field observations of dieldrin, parathion, chlordane and benzene hexachloride have shown these materials cause heavy losses to honey bees. These data indicate that materials found to be more toxic than DDT should be used with extreme caution where bees are concerned and that materials found to be less toxic than DDT may be used with safety."

Table 1.—Effects of Sevin as a contact spray on honey bees.

FORMULATION AND DOSAGE PER 100 GAL.	HOW APPLIED (MACHINE)	NO. HOURS BEFORE 50% OF BEES DIED
Sevin, WP 50%—1 lb.	hydraulic sprayer	0–6
Sevin, WP 50%—1 lb.	air blast	0–6
Sevin, WP 50%—2 lb.	hydraulic sprayer	0–6
Sevin, WP 50%—2 lb.	air blast	0–6
Sevin, M[a] 36%—1 lb.	air blast	0–6
Water spray	air blast	180
Untreated		228

[a] A mull containing 2.5 pounds Sevin per gallon in an oil and emulsifier carrier.

Table 2.—Effects of Am. Cyanamid No. 5223 as a contact spray by air blast machine on honey bees.

DOSAGE AND FORMULATION	PER CENT MORTALITY AFTER 15 DAYS
Am. Cyanamid #5223—1 lb./100 gal.	36.2
Am. Cyanamid #5223—2 lbs./100 gal.	60.2
Water only	36.4
Untreated	36.0

Table 3.—Residual effects of 50% Sevin wettable powder at 1 pound actual in 100 gallons of spray on honey bees.

TREATMENT (MACHINE)	HOURS BETWEEN APPLICATION AND EXPOSURE OF BEES	NO. OF BEES	HOURS TO PRODUCE 50% MORTALITY	COMMENTS
Air blast	0.5	218	0–6	100% dead after 6 hours
Hydraulic	0.5	87	96	
Air blast	24	170	0–6	88.5% dead after 6 hours
Hydraulic	24	227	300	
Air blast	96	194	204	
Hydraulic	96	188	228	
Water, air blast	0.5	221	420	
Untreated		145	276	

Brannon (1958) made the following statement concerning dieldrin: "Dieldrin killed bees up to eight days after application to blooming alfalfa." Apparently the dosage was 0.5 pound actual per acre and both dust and spray formulations were toxic.

METHODS AND PROCEDURE.—The techniques employed were the following. Bees were shaken from brood frames into a pan. They were then placed in sleeve cages, made from aluminum window screening, and taken to the orchard. Cages of bees to be exposed to contact insecticide action were fastened to limbs and the pesticides were applied using the same equipment and practices that a commercial grower would employ. To determine residual effects, the trees were sprayed, the foliage was allowed to dry, and caged bees were then exposed to the residues for 30 minutes. Following the treatments, the cages were brought to the laboratory and the bees were removed to clean holding cages. These were placed in a dark room and the bees were provided with a syrup made of equal parts of sugar and water. Mortalities were observed, usually after 6 hours, and daily thereafter. The data are summarized in tables 1 to 4 inclusive.

DISCUSSION OF RESULTS.—It is obvious that 50% Sevin wettable powder at dosages of 0.5 and 1.0 and the 36% mull formulation at a dosage of 0.36 lb. actual per 100 gallons were highly toxic as contact applications on bees. All three treatments produced more than 50% mortality within 6 hours.

Similar tests with Am. Cyanamid No. 5223 at 1 lb. per 100 gallons resulted in a mortality of 36.2% within 15 days.

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Table 4.—Residual effects of 0.25 pound of actual dieldrin in 100 gallons on honey bees.

Treatment	Hours Between Application and Exposure of Bees	No. of Bees	Hours to Produce 50% Mortality	Comments
Dieldrin	0.5	188	0–24	88.3% dead after 24 hours
Dieldrin	24	200	36	
Dieldrin	96	122	36	
Water only	0.5	221	420	
Untreated		145	276	

This did not differ significantly from the mortality found in the untreated control (36%) or from that shown by bees receiving a water spray (36.4%). At a dosage of 2 lb. per 100 gallons the mortality was 60.2%.

The toxicity of Sevin residues (1 lb. actual per 100 gallons) was found to vary depending on how the applications were made, those from the air blast machine producing greater toxicity. Residues applied with the air blast machine were highly toxic to bees after a 24-hour period. It appeared that after a period of 96 hours the residual toxicity of Sevin to honey bees was reduced.

The toxicity of residues of 0.25 lb. of actual dieldrin per 100 gallons applied by an air blast machine appeared to persist for at least 96 hours so as to produce a mortality of more than 50% within 36 hours after exposure.

The toxicity of Am. Cyanamid No. 5223 to honey bees at the dosages indicated was relatively low.

References Cited

Anderson, L. D., and E. L. Atkins, Jr. 1958. Toxicity of pesticides to honey bees in laboratory and field tests in southern California, 1955–56. Jour. Econ. Ent. 51: 103–8.

Anderson, L. D., and T. O. Tuft. 1952. Toxicity of several new insecticides to honey bees. Jour. Econ. Ent. 45: 465–9.

Brannon, D. H. 1958. Washington Research Progress Report (Entomology). Agric. and Home Econ. Rept. No. 6, 3 pp.

The Occurrence of Insect Parasites of *Choristoneura fumiferana* (Clem.), in Oregon[1]

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Abstract

To date, 34 species of primary parasites have been recovered from the spruce budworm (*Choristoneura fumiferana* (Clem.)) in Oregon. Of these, 15 species are considered of major importance. Analysis of the abundance and distribution of these parasites was based on concepts and methods used in plant sociology.

Within host populations during the period 1953–56, seven species are shown to have increased in density without decreasing in presence, and three species to have increased in presence without decreasing in density. For one infestation, the frequency of parasite species over the area was found to be consistent with density. Whereas most species are believed to be distributed over the region, it is obvious that conditions within a single infestation may be unfavorable to the multiplication of some species.

Secondary parasitization is suggested as a factor retarding the increase in density of primary parasites during the early stages of an outbreak.

Since 1944, outbreaks of the spruce budworm (*Choristoneura fumiferana* (Clem.)) have plagued the forests of Oregon and, to a lesser extent, those of Washington. Protection of the forest resource has required an annual assessment of the need for direct control, as weighed against the current effectiveness of natural control. One of the key factors in natural control is the insect parasite complex attacking the spruce budworm. This paper records the abundance and distribution of parasites of the spruce budworm in Oregon and evaluates their importance.

Data on the occurrence of parasite species have been obtained from two kinds of natural control studies. First, collections have been made at arbitrarily selected locations to determine the representation and abundance of parasite species at a particular time. Second, the overall effectiveness of parasites has been studied in specific infestations annually for correlation with population trend. As a result of these studies, considerable data have been gathered that show the relative importance of these parasite species in many localities in the general area of infestation.

Species of parasites attacking the spruce budworm in British Columbia have been listed by Tothill (1923) and by Wilkes *et al.* (1948) with estimates of per cent parasitization. However, no comprehensive summary has been published previously on parasites of the spruce budworm in Oregon.

Epidemiology.—The spruce budworm was first recorded in the northwestern United States in 1914 near Ashland, Ore. Small centers of infestation were noted during 1927–29 near Northport, Wash.; in 1931 south of Mitchell, Ore.; and during 1941–42 south of Lakeview, Ore. The first significant outbreak occurred during 1943–47 near Chelan, Wash., subsiding in 1948 from natural causes. The current outbreak was first noted in 1944 south of Heppner, Ore. From 1945 to 1949, the number and acreage of epidemic infestations increased rapidly, covering parts of eastern Oregon and eastern Washington, the Oregon and Washington Cascades, and western Oregon. Subsequently, the spruce budworm has been found to

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occur in types containing Douglas-fir and true firs throughout the two states.

In 1949 and continuing until 1955, aerial spray operations were conducted to control this insect where forest values were seriously threatened. Although new infestations have continued to appear in unsprayed areas in eastern Oregon during the period 1949–56, the rate of build-up and spread has slackened. Biotic factors of control have exercised increasing restraint on the multiplication of the spruce budworm, and of these, parasitization by insects has been of major importance.

RECORDS OF PARASITIZATION.—The earliest records of parasitization in this region were obtained by Buckhorn (1941). Further parasite records were obtained from incidental rearings during 1945–47. The first studies of parasitization were made in 1948 by C. F. Speers (1949) at semipermanent plots located in several areas of infestation in eastern Oregon. Parasite records were obtained in western Oregon by K. H. Wright (1949) in connection with control operations, and in eastern Oregon by W. D. Buchanan (1949) during mass collections for parasite species to be liberated in the Northeast. In 1950, biological studies of the spruce budworm were begun, with emphasis on the role of its parasites. These studies have been continued without interruption and preliminary findings have been reported by Carolin (1950), Carolin & Wright (1953), and Coulter (1953).

To date, 34 species of primary parasites have been recovered from the spruce budworm in Oregon and Washington. It is believed that all parasites of major importance and a majority of those of minor importance have been detected. Additional minor parasites will probably be discovered as studies progress.

APPRAISAL OF THE RELATIVE IMPORTANCE OF PARASITE SPECIES.—To analyze the records on parasitization, the following procedure was used: First, the various parasite species were placed in groups according to their occurrence during the life cycle of the host. Local abundance for each species within a natural group, as expressed by per cent parasitization, was then translated into classes to simplify the data. Distribution over a limited area was determined by listing presence or absence for each species at selected localities within this area. Distribution over a large geographical area was determined by listing presence or absence for each species at all localities at which records were obtained. Finally, the records were grouped by periods of time in order to determine trends for these species.

The great majority of parasites attacking the spruce budworm fall into readily distinguished natural groups. Dowden *et al.* (1951) have defined five groups according to the period in which the host is attacked, as follows: Eggs, hibernating larvae, small larvae in the opening buds (third, fourth, fifth stadia), large larvae (full-grown larvae in the sixth stadium), and pupae. To obtain a reliable measurement of the effect of parasite species comprising each attack group, collection of host material must follow the period of attack and yet precede the time at which the species of a group begin to abandon the host. Despite observance of such timing, estimates of the effect of a parasite species may vary greatly between localities and from year to year at the same locality.

Per cent parasitization is used by the authors as a measure of density where the host constitutes the environment or territory in which the parasites are distributed. Density is by definition a quantitative measure of abundance (Cain 1932) and distribution is interpreted through two concepts used in phytosociological studies—frequency and presence. The uniformity of distribution of a parasite species within a single infestation is described by the concept of frequency. Frequency is a measure of occurrence within a community and is defined as the percentage of the total number of sample plots in which a given species is found. A related concept, called presence, is used to express the degree of regularity with which a species reoccurs in examples of different infestations. Presence is defined as the percentage of the total number of stands in which a given species is found.

Annual records at permanent plots would provide the best basis for expressing trends in density, frequency, and presence. However, the localities from which records could be obtained varied from year to year, due chiefly to an assiduously pursued control program. The division of plot records according to periods of time awaited tabulation of the data and was destined to be broad and arbitrary.

TABULATION AND GROUPING OF DATA.—File cards were prepared for each species of parasite, and all collections made at the proper time for its recovery were listed. For each collection, the life stages of the host, number of hosts obtained, and number of hosts killed by the parasite were shown. (Small larvae were dissected to obtain data on parasites of hibernating larvae; other stages were reared for parasites.) Since parasite grouping was based on time of attack, hosts containing parasites belonging to a preceding group were dropped from the count, and a corrected number of hosts obtained. Per cent parasitization was then calculated to determine density class for each collection. The percentage of all collections in which the parasite was recovered was used to show presence. In only one area were sufficient collections obtained from a single infestation to show frequency.

Five classes of density were arbitrarily selected for use: D. 1—any positive figure up to and including 1.5% parasitization; D. 2—from 1.6 to 5.5% parasitization; D. 3—from 5.6 to 10.5%; D. 4—from 10.6 to 25.5%; and D. 5—more than 25.5%. As recommended by Braun-Blanquet (1932), five classes of frequency and presence were used with each class consisting of a 20% interval. For frequency, these classes are identified as A (1–20%) to E (81–100%); and for presence, Pr. 1 (1–20%) to Pr. 5 (81–100%).

In an attempt to detect trend in density and presence, an average density class and average presence class were calculated for each species for two periods within the outbreak. Periods were selected for which the number of records were approximately equal: 1948–1952 and 1953–56. To show trends clearly, average density class was expressed to one decimal place for values above 1.0.

It is believed that parasite species that occur occasionally or consistently in density classes above D. 1 can be reliably catalogued as to density and presence. At times and in specific localities they may be considered important species. Data on these species are presented in table 1. Species occurring only in D. 1 cannot be classified as to presence or density with accuracy. These species are

Table 1.—The occurrence of major insect parasites of the spruce budworm in Oregon.

Species	Host Stages[a]		1948–52			1953–56			Trend Observed[b]	
				Average			Average			
	At-tacked	Killed	No. of Collec-tions	Den-sity (Class)	Pres-ence (Class)	No. of Collec-tions	Den-sity (Class)	Pres-ence (Class)	Den-sity	Pres-ence
HYMENOPTERA: Braconidae										
Apanteles fumiferanae	II	IV, V	23	3.5	5	14	4.2	5	>	—
Eubadizon gracile	V, VI	VI	22	1.0	1	22	1.5	2	>	>
HYMENOPTERA: Ichneumonidae										
Ephialtes ontario (Cress.)	pupae	pupae	12	1.2	2	17	1.2	2	—	—
Glypta fumiferanae	II	VI	28	3.0	5	14	4.0	5	>	—
Horogenes sp.	II	V	24	1.5	4	14	1.6	4	—	—
Itoplectis 4-cingulatus	pupae	pupae	12	1.2	5	17	1.3	4	—	<
Phaeogenes hariolus	pupae	pupae	12	2.0	4	17	1.9	5	—	>
Phytodietus fumiferanae	VI	VI	23	1.3	4	22	1.8	5	>	>
HYMENOPTERA: Trichogrammatidae										
Trichogramma minutum	eggs	eggs	16	2.0	3	15	1.6	4	<	>
DIPTERA: Larvaevoridae										
Ceromasia auricaudata	VI	pupae	24	1.5	4	22	2.6	5	>	>
Lypha setifacies	V, VI	VI	22	1.0[c]	1[c]	22	1.0[c]	1	—	—
Madremyia saundersii	VI	VI, pupae	24	1.7	5	22	3.4	5	>	—
Omotoma fumiferanae	VI	pupae	24	2.0	5	22	2.8	3	>	<
Phorocera incrassata	VI	pupae	24	1.4	2	22	1.7	4	>	>
DIPTERA: Sarcophagidae										
Agria affinis	pupae[d]	pupae	11	1.8	4	17	2.1	5	—	>

[a] Larval stadium indicated by Roman numerals.
[b] Symbols denote the following trends: > increasing, < decreasing,—no trend evident.
[c] Records only for eastern Oregon; for western Oregon, 3 collections prior to spraying showed average density of 3.7 and presence class of 5.
[d] Rarely attacks sixth stadium larvae, causing a mummified appearance of the host.

considered of little economic importance and are listed separately in table 2.

IMPORTANCE OF SPECIES WITHIN ATTACK GROUPS.—The overall effect of three hymenopterous species attacking hibernating larvae has been relatively great throughout the outbreak period. *Apanteles fumiferanae* Vier. and *Glypta fumiferanae* (Vier.) have been recorded consistently in density classes 3 and 4; *Horogenes* sp., consistently in classes 1 and 2. All three species are present throughout the region. The species of external parasites reported attacking hibernating larvae in other regions have not been found in Oregon infestations.

Small larvae in the opening buds are attacked by a few species of Hymenoptera. Externally feeding chalcidoids are occasionally found but are difficult to rear successfully to the adult stage. Of these, only *Elachertus cacoeciae* How. has been definitely recovered. One record was obtained for the ichneumonid *Scambus ?hispae* (Harr.) which killed a fourth stadium larva. Although eggs of the ichneumonid *Phytodietus fumiferanae* Rohw. have been found occasionally on fourth and fifth stadia larvae, it is doubtful that the size of the host permits the parasite to complete development. Eggs of the internal parasite *Eubadizon gracile* Prov. have been found in fourth stadium larvae, but attack normally occurs later.

Large larvae are attacked by at least eight species of Hymenoptera and 10 species of Diptera. The combined effect of these species is often considerable. Only one hymenopterous parasite, *Phytodietus fumiferanae*, has been of importance, occurring at a relatively low density but generally present. Five larvaevorids (Diptera) have been of considerable importance. During 1952–56 two of these species, *Madremyia saundersii* (Will.) and *Ceromasia auricaudata* T.T., occurred in density class 3 and in presence class 5. As the outbreak has progressed, *Phorocera incrassata* Smith has been recorded more often and at gradually increasing density. On the other hand, *Omotoma fumiferanae* (Toth.) has been recorded less often as the outbreak has progressed, although at moderate density (D. 3) when present. *Lypha setifacies* (West) is of little importance in eastern Oregon, but collections in 1951 showed it was of moderate density in western Oregon infestations.

Pupae are attacked by seven species of Hymenoptera and one species of Diptera. The combined effect of these parasites is normally small, however. An ichneumonid, *Phaeogenes hariolus* (Cress.), and a sarcophagid, *Agria affinis* (Fall.), are of chief importance, occurring on the average in density class 2 and with high presence. A second ichneumonid, *Itoplectis 4-cingulatus* (Prov.), is normally present but at a low density.

The only egg parasite recovered has been *Trichogramma minutum* Riley. In this region, the species has not been significant in the control of spruce budworm populations. It is recovered sporadically, often at a very low density. The highest recorded parasitization was 8% of the eggs.

REGIONAL TRENDS IN DENSITY AND PRESENCE OF PARASITE SPECIES.—It has been occasionally noted that a species of parasite recovered with little difficulty at the start of a general outbreak will become extremely rare as

the outbreak progresses. An example is *Exochus* sp. in spruce budworm outbreaks in Maine (Jaynes & Drooz 1952). Similar evidence was obtained from a single collection in Oregon in 1945, when four species of pupal parasites were reared. Two of these have not been subsequently recovered, a third species recovered only once, and a fourth species recovered twice (table 2).

The division of records for the outbreak period into two segments points out trends in the occurrence of the more common parasite species. Compared with 1948–52, seven species increased significantly in density without decreasing in presence during 1953–56. These were *Apanteles fumiferanae, Glypta fumiferanae, Eubadizon gracile, Phytodietus fumiferanae, Ceromasia auricaudata, Madremyia saundersii,* and *Phorocera incrassata.* Three species—*Phaeogenes hariolus, Agria affinis,* and *Meteorus trachynotus* Vier.—have shown higher presence, and three species—*Aplomya caesar* (Ald.), *Omotoma fumiferanae,* and *Itoplectis 4-cingulatus*—have declined in presence. None of the major species have declined in density.

The net result of these changes has been an increased effect of parasites, particularly those attacking hibernating larvae and larvae in the sixth stadium, as the outbreak has progressed. Long-term research studies at permanent study plots are showing a gradual increase in parasitization from year to year up to the point where an infestation collapses. At that point it appears that per cent parasitization may be reduced, although not drastically.

LOCAL DISTRIBUTION OF PARASITE SPECIES.—In 1957, data were obtained to show the distribution and density of parasite species attacking sixth stadium larvae within an infestation covering 10,000 acres. The locations for sample plots were arbitrarily selected on a map, and a fixed-size foliage sample was taken at 20 points to obtain spruce budworm. Each of these collections was handled separately for a determination of per cent parasitization by species.

Seven species of parasites were recovered. The frequency percentage found for each of three major species (*Ceromasia auricaudata, Madremyia saundersii,* and *Phytodietus fumiferanae*) was in direct proportion to their respective densities for the area. The direct relation of frequency to density was approximated in the case of four species of minor importance. Although elevation, exposure, and population level varied considerably for points at which collections were obtained, little variation in distribution of parasite species was found that could not be attributed to number of insects collected. Table 3 shows the frequency and density records obtained.

VAGARIES OF PARASITE DISTRIBUTION.—It may be assumed that most parasite species recovered during these studies are distributed throughout the state. However, conditions in specific infestations may not be conducive to the multiplication of certain species, hence these species are not often recovered. The disparity in density and presence of *Lypha setifacies* between western Oregon and eastern Oregon infestations has already been mentioned.

Table 2.—Insect parasites of the spruce budworm of minor importance in Oregon.

SPECIES	HOST STAGES[a]		NUMBER OF COLLECTIONS			
			1948–52		1953–56	
	Attacked	Killed	Total	With Recoveries	Total	With Recoveries
HYMENOPTERA: Braconidae						
Agathis sp.	?	VI	22	0	22	1
Bracon politiventris (Cush.)	VI	VI	25	4	22	3
Meteorus trachynotus	V, VI	VI	22	1	22	5
Oncophanes americanus[b]	V, VI	V, VI	22	1	22	0
HYMENOPTERA: Ichneumonidae						
Coccygomimus pedalis (Cress.)[c]	pupae	pupae	12	0	17	0
Exochus sp.[b]	VI	pupae	23	2	22	0
Horogenes n. sp.[b]	?	VI	22	1	22	0
Ischnus inquisitorius atriceps (Cress.)	?	pupae	12	1	17	0
Itoplectis viduata (Grav.)[c]	pupae	pupae	12	0	17	0
Labrorhychus sp.[d]	VI	pupae	22	1	22	0
Scambus alboricta (Cress.)	VI	VI	22	1	22	0
Scambus ?hispae	?	IV	23	0	15	1
Theronia atalantae (Poda)[d]	pupae	pupae	12	1	17	1
HYMENOPTERA: Elachertidae						
Elachertus cacaeciae	V, VI	V, VI	25	1	22	0
DIPTERA: Larvaevoridae						
Actia interrupta Curr.[e]	V, VI	VI	21	5	22	4
Aplomya caesar	VI	VI, pupae	24	9	22	2
Nemorilla floralis Fall.	VI	pupae	24	2	23	2
Phorocera erecta Coq.	VI	pupae	24	3	22	3
Phryxe pecosensis T.T.	VI	VI, pupae	24	5	22	2

[a] Larval stadium indicated by Roman numerals.
[b] Recovered in Western Oregon only.
[c] Recovered in 1945 only.
[d] Also recovered in 1945.
[e] Common in sprayed areas.

Table 3.—Frequency and density of seven insect parasites of the spruce budworm based on 20 points in the Bald Ridge infestation near Baker, Oregon, in 1957.

		SPECIES OF PARASITES RECOVERED[a]						
		Species Recovered						
Degree of Infestation and Plot Number	No. of Hosts	*Aplomya caesar*	*Meteorus trachynotus*	*Bracon politiventris*	*Phorocera incrassata*	*Phytodietus fumiferanae*	*Madremyia saundersii*	*Ceromasia auricaudata*
Light								
1	11	—	—	—	—	—	—	—
2	9	—	—	—	—	X	—	X
3	8	—	—	X	—	X	—	X
4	8	—	—	—	—	—	—	X
5	17	—	—	X	—	—	—	X
6	8	—	—	—	—	—	—	—
7	4	—	X	X	—	—	—	—
8	4	—	—	—	—	—	—	—
Moderate								
9	35	X	—	—	X	—	X	X
10	47	—	—	—	—	X	X	X
11	54	—	—	—	X	—	X	X
12	32	—	—	—	—	—	—	X
13	40	—	X	—	—	X	X	X
14	30	—	—	—	—	—	—	X
15	33	—	—	—	—	—	X	X
16	41	—	—	—	X	—	X	—
17	28	—	X	—	—	X	—	X
18	29	—	—	—	—	—	—	X
Heavy								
19	73	—	—	—	—	—	X	X
20	68	—	—	—	—	X	X	X
No. of records		1	3	3	3	6	8	15
No. of hosts killed		1	3	3	6	13	46	110
Frequency per cent (20 plots)		5.0	15.0	15.0	15.0	30.0	40.0	75.0
Density per cent (579 hosts)		0.2	0.5	0.5	1.0	2.2	7.9	19.0

[a] "X" indicates parasites present.

Two other species, *Exochus* sp. and *Oncophanes americanus* (Weed), reared from host larvae in western Oregon have not been found during the more intensive work in the eastern part of the state. In the isolated infestation for which frequency estimates were obtained, not one specimen of *Omotoma fumiferanae* has been recovered in three seasons of collecting at 20 scattered points. Furthermore, collections at a permanent study plot in a second area of infestation did not yield *Ceromasia auricaudata* until the fifth year of study.

It becomes obvious that variation in the presence of parasite species occurs with time as well as place of infestation. Therefore, the true density and presence of parasite species are reflected accurately only by collections taken from several infestations over a period of years.

SECONDARY PARASITES AS A FACTOR IN THE DENSITY OF PRIMARY PARASITES.—Secondary parasitization is often a significant factor in limiting the effectiveness of primary parasites. Evaluation of the importance of this factor is extremely difficult. Pertinent data for the Oregon spruce budworm epidemic are limited and mainly qualitative in nature.

Two primary parasites attacking hibernating larvae are rather consistently attacked by other parasites. Approximately 25% of the field-collected cocoons of *Apanteles fumiferanae* and 30 to 60% of those of *Horogenes* sp. regularly produce secondary parasites. Secondary parasitization of *Glypta fumiferanae*, however, has been very low.

Primary parasites attacking full-grown larvae show varying degrees of secondary parasitization. Since 1951, puparia of larvaevorids have shown a very low parasitization, almost entirely from the chalcidoid *Brachymeria compsilurae* (Cwfd.). Parasitization of material collected from 1948 to 1951 appeared to be somewhat higher, and one collection of pupae during 1941 produced *Brachymeria* but no primary parasites. Hymenopterous parasites of full-grown larvae have been attacked to a greater degree. The incidence of attack of *Phytodietus fumiferanae*, due to the lengthy period spent in the cocoon, is fairly high; probably a third of the cocoons produce hyperparasites. *Meteorus trachynotus*, noted for its rapid buildup in budworm infestations in the Northeast, was found in increased numbers in Oregon in 1955. However, most of the field-collected cocoons produced secondary parasites, and *Meteorus* populations the following year were exceedingly low. A similar pattern was found for *Eubadizon gracile* during the same period.

Hymenopterous parasites attacking pupae were rarely attacked by other parasites, except in localities where the density of these pupal parasites was much above the regional average. *Amblymerus verditer* (Nort.), a gregarious chalcidoid, was the major secondary parasite recovered.

It appears that secondary parasites exert a considerable restraining effect on primary parasites during the early

stages of an outbreak. Secondary parasitization may be a cause of static densities of primary parasites, noted to occur early during outbreaks in Oregon. However, this effect is temporary since primary parasites are eventually released by their greater reproductive capacities.

REFERENCES CITED

Braun-Blanquet, J. 1932. Plant sociology. New York and London.

Buchanan, W. D. 1949. Spruce budworm report. U. S. Bur. Ent. and Plant Quar., special report, Fort Collins, Colo. (unpublished.)

Buckhorn, W. J. 1941. Unpublished data. Pacific NW. Forest and Range Expt. Sta., Forest Service, Portland, Ore.

Cain, S. A. 1932. Concerning certain phytosociological concepts. Ecol. Monogr. **2**: 475–508.

Carolin, V. M. 1950. Studies of the biological control of the spruce budworm in Oregon, 1950. U. S. Bur. Ent. and Plant Quar., special report, New Haven, Conn. (unpublished.)

Carolin, V. M., and K. H. Wright. 1953. Studies of the biology and ecology of the spruce budworm in Oregon and Washington, 1951. U. S. Bur. Ent. and Plant Quar., special report, Portland, Ore. (mimeographed.)

Coulter, W. K. 1953. Studies of the biology and control of the spruce budworm in Oregon and Washington, 1952. U. S. Bur. Ent. and Plant Quar., special report, Portland, Ore. (mimeographed.)

Dowden, P. B., V. M. Carolin, and C. O. Dirks. 1951. Natural control factors affecting the spruce budworm in the Adirondacks during 1946–48. Jour. Econ. Ent. **43**: 774–83.

Jaynes, H. A., and A. T. Drooz. 1952. The importance of parasites in the spruce budworm infestations in New York and Maine. Jour. Econ. Ent. **45**: 1057–61.

Speers, C. F. 1949. Studies of biological control factors affecting the spruce budworm. Division of Forest Insect Investigations Annual Report for 1948: 44–46 New Haven, Conn. (unpublished.)

Tothill, J. D. 1923. Notes on the outbreaks of the spruce budworm, forest tent caterpillar, and larch sawfly in New Brunswick. Proc. Acadian Ent. Soc. **1922**(8): 172–82.

Wilkes, A., H. C. Coppel, and W. G. Mathers. 1948. Notes on the insect parasites of the spruce budworm *Choristoneura fumiferana* (Clem.) in British Columbia. Canadian Ent. **80**(1–12): 138–55.

Wright, K. H. 1949. Unpublished data. Pacific NW. Forest and Range Expt. Sta., Forest Service, Portland, Ore.

Rearing and Treating Successive Generations of Boll Weevils[1]

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ABSTRACT

In studies of boll weevil (*Anthonomus grandis* Boh.) resistance to chlorinated hydrocarbon insecticides, 10 generations were reared from September 1956 to September 1958 and 4 to 6 generations were treated topically with insecticides. There was no indication of reversion to susceptibility in the untreated strains when tested with endrin. Endrin applied to resistant and susceptible strains increased the LD-50 and LD-90, and gave some changes in slope of lines. A mixture of equal parts of endrin and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) and Guthion alone gave no changes in LD-50, LD-90, or slope. However, this does not prove that resistance will not develop with continued selection in large populations of boll weevils.

In 1955 it was shown that the boll weevil, *Anthonomus grandis* Boh., had developed a high degree of resistance to chlorinated hydrocarbon insecticides (Roussel & Clower 1955). Since this posed a serious threat to the economical production of cotton throughout the boll-weevil belt, a study of this problem was undertaken at Baton Rouge, La., in 1956.

The objectives were to determine (1) how many generations are required to lose resistance to a chlorinated hydrocarbon after it has been acquired; (2) how many generations are required to acquire resistance to a chlorinated hydrocarbon; (3) whether resistance will develop as rapidly, if at all, when the boll weevils are exposed simultaneously to two groups of insecticides such as a chlorinated hydrocarbon and an organic-phosphorus compound; (4) whether boll weevils already resistant to chlorinated hydrocarbons will become resistant to an organic-phosphorus compound and, if so, how many generations will be required; (5) whether boll weevils not resistant to chlorinated hydrocarbons will become resistant to a phosphorus compound and, if so, how many generations will be required; (6) the degree to which boll weevils have already developed resistance to a chlorinated hydrocarbon and whether the resistance increases in subsequent generations.

Two groups of boll weevils were used to start this study—a susceptible group (S) from Baton Rouge and a resistant group (R) from Tensas Parish. Each group was subjected to treatments of the chlorinated hydrocarbon endrin (S-E and R-E), the organic-phosphorus compound Guthion (®*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate) (S-G and R-G), equal parts of endrin and Guthion (S-EG and R-EG), or untreated (S-U and R-U).

Cages 12 by 8 feet and 5 feet high covered with 16-mesh aluminum wire were used to confine the boll weevils on fruiting cotton plants in the field. Untreated weevils were introduced into cages to start the R-U and S-U strains. Some of the weevils from the untreated strains of each generation were treated topically with endrin to determine dosage-mortality lines. Survivors from topical tests were introduced into cages containing all other strains 48

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Table 1.—Rearing and dosage-mortality data for each generation of resistant boll weevils, September 1956 to September 1958.

Generation[a]	Date Emerged	Number of Weevils Emerged	Number of Weevils Treated	LD-50 (Mg./Gm.)	LD-90 (Mg./Gm.)	Slope
Untreated (R-U)						
	1956					
Initial	—	—	174	0.018	0.314	1.04
1st	9/26–10/20	239	144	.071	8.710	.61
	1957					
2nd[a]	5/ 3– 5/17	22	—	—	—	—
3rd	5/27– 6/17	65	—	—	—	—
4th	6/22– 7/27	291	210	.131	4,790.000	.28
5th	7/24– 8/15	190	116	.033	3.930	.62
6th	8/29– 9/24	38	—	—	—	—
	1958					
7th[a]	—	0	—	—	—	—
8th	7/ 2– 7/18	41	—	—	—	—
9th	7/30– 8/21	59	42	16.742	683.998	.80
10th	8/31– 9/18	131	—	—	—	—
Treated with Endrin (R-E)						
	1956					
Initial	—	—	174	0.018	0.314	1.04
1st	10/ 1–10/20	262	161	6.440	984.000	.59
	1957					
2nd[a]	5/ 3– 5/11	16	—	—	—	—
3rd	5/27– 6/16	111	—	—	—	—
4th	6/21– 7/28	370	300	.299	3.690	1.17
5th	7/28– 8/13	76	—	—	—	—
6th	8/29– 9/24	31	—	—	—	—
	1958					
7th[a]	4/18– 5/ 9	3	—	—	—	—
8th	6/30– 7/14	37	—	—	—	—
9th	7/28– 8/23	73	67	.968	5.888	1.63
10th	8/31– 9/18	179	169	539.400	3,917,000.000	.33
Treated with Endrin and Guthion (R-EG)						
	1956					
Initial	—	—	211	0.006	0.012	4.33
1st	10/ 1–10/20	322	212	.005	.019	2.35
	1957					
2nd[a]	4/20– 5/24	16	—	—	—	—
3rd	5/29– 6/19	53	—	—	—	—
4th	6/24– 7/24	111	78	.005	.011	4.38
5th	7/28– 8/15	69	36	.007	.010	6.54
6th	8/28– 9/23	68	—	—	—	—
	1958					
7th[a]	—	0	—	—	—	—
8th	7/ 2– 7/14	24	—	—	—	—
9th	7/28– 8/19	106	90	.006	.013	3.98
10th	8/29– 9/17	121	105	.009	.020	3.76
Treated with Guthion (R-G)						
	1956					
Initial	—	—	138	0.002	0.010	1.97
1st	10/ 1–10/20	273	177	.004	.019	1.78
	1957					
2nd[a]	5/ 3– 5/20	15	—	—	—	—
3rd	5/28– 6/17	104	—	—	—	—
4th	6/24– 7/24	255	200	.003	.007	3.22
5th	7/28– 8/ 9	57	40	.003	.004	7.98
6th	8/29– 9/17	56	—	—	—	—
	1958					
7th[a]	5/ 5– 5/15	3	—	—	—	—
8th	6/30– 7/14	28	—	—	—	—
9th	7/28– 8/23	33	—	—	—	—
10th	8/29– 9/18	66	54	.003	.005	5.29

[a] Overwintered generations.

hours or longer after insecticide application. One cage was used for each strain of each generation except the overwintered generation, for which three cages were used. To start the first and each succeeding generation about 12 pairs of weevils were introduced into each cage. These weevils were selected at random from the survivors of the various dosages. To all the generations reared in 1957 two pairs of weevils from the original strains were added in order to reduce the degree of inbreeding. The fallen punctured cotton squares were collected from the ground in the cages three times each week and placed in small rearing cages in an air-conditioned laboratory. The emerging weevils were fed on either squares or squares and blooms for 48 hours or longer before they were given topical applications of the insecticides.

Weather conditions during the winter of 1957–58 were unfavorable for boll weevil survival. Only five weevils survived in the S-EG cages; three each in R-E, R-G, and S-E; two in S-U; one in S-G; and none in R-U and R-EG. Since there were no survivors in the large cages where the original groups of weevils were maintained, it was necessary to obtain weevils from other sources to replace those groups that failed to survive the winter. Resistant boll weevils from Tallulah were introduced into the R-U, R-E, R-EG, and R-G cages on June 10, with the result that R-U and R-EG were new strains started in 1958 and R-E and R-G were mixtures of the 1956 and 1957 strains and Tallulah weevils. It was about 1 month later before susceptible weevils were available for use. On July 8 susceptible weevils from Clinton were introduced into the S-U, S-E, and S-G cages. S-EG is the only pure strain of the original started in 1956.

Table 2.—Rearing and dosage-mortality data for each generation of susceptible boll weevils, September 1956 to September 1958.

Generation[a]	Date Emerged	Number of Weevils Emerged	Number of Weevils Treated	LD-50 (Mg./Gm.)	LD-90 (Mg./Gm.)	Slope
Untreated (S-U)						
	1956					
Initial	—	—	176	0.001	0.005	2.21
1st	9/28–10/19	269	153	.006	.020	2.62
	1957					
2nd[a]	4/15– 5/31	59	—	—	—	—
3rd	5/22– 6/16	20	—	—	—	—
4th	6/20– 7/24	196	116	.005	.014	2.67
5th	7/27– 8/13	138	30	.073	.921	1.16
6th	8/27– 9/23	27	—	—	—	—
	1958					
7th[a]	4/25– 5/13	2	—	—	—	—
8th	—	0	—	—	—	—
9th	7/28– 8/25	69	—	—	—	—
10th	8/31– 9/19	81	47	.007	.021	2.80
Treated with Endrin (S-E)						
	1956					
Initial	—	—	176	0.001	0.005	2.21
1st	10/ 1–10/20	331	209	.021	.178	1.38
	1957					
2nd[a]	4/18– 5/31	56	—	—	—	—
3rd	5/22– 6/17	37	—	—	—	—
4th	6/23– 7/28	359	251	.021	.217	1.26
5th	7/28– 8/11	73	43	.098	.574	1.67
6th	8/27– 9/19	121	92	.027	.078	2.78
	1958					
7th[a]	4/29– 5/12	3	—	—	—	—
8th	—	0	—	—	—	—
9th	7/30– 8/25	48	46	.034	.309	1.34
10th	8/31– 9/19	59	50	.112	3.900	.83
Treated with Endrin and Guthion (S-EG)						
	1956					
Initial	—	—	229	0.005	0.016	2.49
1st	10/ 1–10/20	352	239	.005	.013	3.34
	1957					
2nd[a]	4/18– 5/31	71	—	—	—	—
3rd	5/23– 6/17	23	—	—	—	—
4th	6/23– 7/25	316	232	.003	.006	4.17
5th	7/28– 8/ 7	63	28	.005	.008	5.54
6th	8/26– 9/23	100	63	.003	.005	3.92
	1958					
7th[a]	4/23– 5/26	5	—	—	—	—
8th	6/30– 7/18	39	—	—	—	—
9th	7/28– 8/25	86	62	.006	.016	2.96
10th	8/31– 9/19	94	87	.006	.009	6.82
Treated with Guthion (S-G)						
	1956					
Initial	—	—	232	0.001	0.010	1.46
1st	10/ 2–10/20	371	265	.003	.008	3.51
	1957					
2nd[a]	4/18– 5/31	50	—	—	—	—
3rd	5/20 –6/13	22	—	—	—	—
4th	6/23– 7/25	336	246	.001	.009	.84
5th	7/27– 8/11	68	37	.002	.003	4.63
6th	8/26– 9/14	52	27	.004	.007	4.38
	1958					
7th[a]	5/12–	1	—	—	—	—
8th	—	0	—	—	—	—
9th	7/28– 8/25	90	74	.003	.008	3.17
10th	8/31– 9/19	139	127	.002	.006	3.50

[a] Overwintered generations.

Table 1 gives the rearing data, number treated, LD-50, LD-90, and slope for each generation of the resistant strains. Table 2 gives similar data for the susceptible strains.

Ten generations were reared from September 1956 to September 1958. From four to six generations were treated topically with insecticides. There was no indication of reversion to susceptibility in the untreated strains when tested with endrin. Endrin applied to resistant and susceptible strains increased the LD-50 and LD-90, and gave some changes in slope of lines. A mixture of equal parts of endrin and Guthion and Guthion alone gave no changes in LD-50, LD-90, or slope. However, this does not prove that resistance will not develop with continued selection in large populations of boll weevils.

REFERENCE CITED

Roussel, J. S., and D. F. Clower. 1955. Resistance to the chlorinated hydrocarbon insecticides in the boll weevil (*Anthonomus grandis* Boh.). Louisiana Agric. Expt. Sta. Circ. **41.**

Control of Insects with Pyrethrum Sprays in Wheat Stored in Ships' Holds[1]

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ABSTRACT

The use of pyrethrum sprays applied to the surfaces of reserve wheat stored in surplus ships at periodical intervals during 1957 in lieu of the previous method of fumigating the wheat was carefully evaluated in relation to the suppression of stored-grain insect infestations at the James River and Hudson River Reserve Fleets.

At the James River Fleet, a spray containing 0.3% of pyrethrins and 3.0% of piperonyl butoxide, applied at the rate of 2 quarts per 1,000 square feet of grain, ship skin, bulkhead, and underside tween deck surface, five or more times from April through July, suppressed both moth and beetle infestations to a very low population level. After July both moths and beetles began to increase. The pyrethrum spray was supplemented by a methoxychlor residual spray applied to the nonstorage sections of the ships, but most of the suppression was accomplished by the pyrethrum spray.

At the Hudson River Fleet four periodical applications of the same spray used at the James River Fleet almost completely suppressed both moth and beetle infestations.

The Commodity Credit Corporation has for several years used ships in the Reserve Fleets as storages for reserve stocks of government-owned wheat. On the East coast, two floating storage sites are maintained, one at the James River Reserve Fleet, located at Ft. Eustis, Va. (fig. 1), and the second at the Hudson River Reserve Fleet, located at Jones Point, N. Y. Liberty ships are used, and about 225,000 bushels are stored in each ship. The grain is stored in the lower holds only, and is carefully leveled off approximately 4 feet below the tween deck. The hatch opening between the lower hold and the tween deck is left uncovered, but the main hatchway on the weather deck is covered, with appropriate ventilators installed along the sides of the hatch covers. The features of these storages, the capacities of the various holds, and other pertinent facts were discussed in a previous article (Phillips 1955).

The control of insects in ship-stored wheat at these sites has been a considerable problem, especially at the James River Fleet. The Indian-meal moth (*Plodia interpunctella* (Hbn.)) has been the most troublesome insect, followed by the granary weevil (*Sitophilus granarius* (L.)), rice weevil (*Sitophilus oryza* (L.)), flour beetles (*Tribolium* spp.), saw-toothed grain beetle (*Oryzaephilus surinamensis* (L.)), and occasionally psocids. All holds in both fleets used for grain storage are equipped with aeration systems, and the bulk of the wheat remains at temperatures ranging from 60° down to 32° F. the year around as a result of extensive cooling during the winter season. Insect infestation is mostly confined to the top 5 or 10 feet of each hold where the grain responds to outside summer temperatures and warms up to that level. Until the 1957 season, fumigation had been the principal control measure.

In the 1956 season, a pyrethrum spray was applied to the grain surface in each hold, to supplement the fumigation treatment, with the objective of preventing reinfestation by insects from the nonstorage areas in the ships. In addition, at the James River Fleet, eight ships on which moth populations had built up to high numbers following a fumigation were given a second application of the pyrethrum spray. The spray was applied not only to the surface of the grain, but also to the exposed skin of the ship and bulkheads in the lower hold, and the underside of the tween deck. The control and subsequent suppression of the moth populations was so pronounced that in the 1957 season routine fumigation of all holds was discontinued

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[2] This is one of the laboratories of the Stored-Product Insects Section, Biological Sciences Branch, Marketing Research Division, Agricultural Marketing Service, U. S. Department of Agriculture.

[3] The Grain Fleet Supervisors at each fleet, C. W. Bovard of the James River Fleet, and David J. Ryan of the Hudson River Fleet, cooperated fully in these studies so that accurate and complete records could be taken and a thorough evaluation made of the program. At the James River Fleet Z. R. Burnett, A. R. Keith, J. R. Morgan, J. S. Parks, H. A. Phillips, and M. D. Treadway, under the supervision of N. E. Arehart, inspected the holds and recorded the insects found. At the Hudson River Fleet this task was carried out by Joe Burkert, Bob Cusack, Walter Gorton, Vince Kenney, Lester Prosynchak, Louis Provanzana, and Phillip Wade under the supervision of Raymond L. Battagliola.

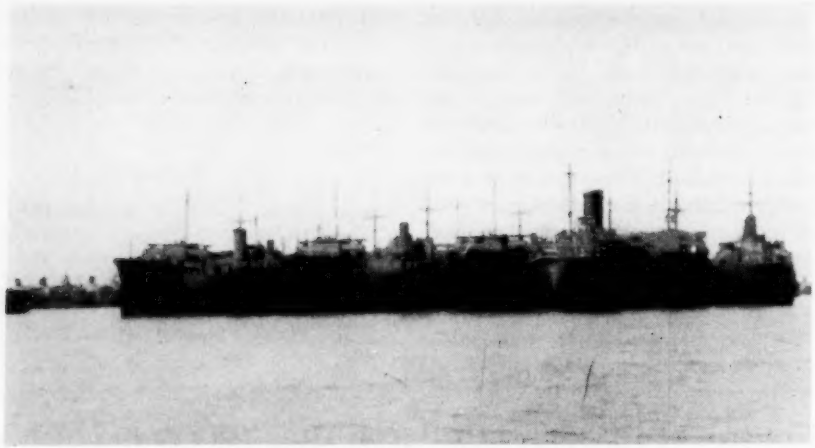

FIG. 1.—View of ships in the James River Reserve Fleet used for storage of wheat.

and pyrethrum sprays were applied periodically in an attempt to control moth infestations. It was intended that as subsurface beetle populations (weevils or bran bugs) became evident or, if the moth infestations got out of hand, the particular holds showing infestation would be fumigated.

The Grain Fleet Supervisors at each fleet provided the Stored-Grain Insects Laboratory with enough assistants so that the insect populations could be counted in every hold twice monthly, in order to evaluate the success of this venture. This paper presents the results of the insect survey and an evaluation of the treatments used.

PROCEDURE.—A pyrethrum spray made from an emulsifiable concentrate of pyrethrins and piperonyl butoxide in 1 to 10 ratio, so that the dilute spray contained 0.3% pyrethrins and 3.0% piperonyl butoxide, was applied at a minimum rate of 2 quarts per 1,000 square feet of surface to the grain surface in each hold, to the exposed skin of the ship and bulkheads above the grain in the lower hold, and to the underside of the tween deck. The sprayed space totaled 55,000 square feet in a typical Liberty ship. All treatments were applied by commercial pest control operators.

At the James River Fleet most ships (except those withdrawn for unloading) received five applications, a few received six, and one received seven. At the Hudson River Fleet all ships received four applications. The spray schedule was as follows:

Application	*James River*	*Hudson River*
First	April 1–15	May 15–31
Second	April 16–30	June 1–15
Third	May 1–31	June 16–July 15
Fourth	June 1–30	July 16–Aug. 15
Fifth	July 1–31	
Sixth	Aug. 1–Sept. 15	
Seventh	Sept. 1–30	

To supplement the pyrethrum spray, a 3% methoxychlor residual spray made from an emulsifiable concentrate was applied at the minimum rate of 1½ gallons per 1,000 square feet of surface to the skin of the ship, the deck floor of the tween deck hold, the underside of the weather deck, and to other nonstorage areas, such as escape trunks and mast houses, which totaled 64,900 square feet per ship. This spray was applied only once—during May to the James River ships, and between June 16 and July 15 to the Hudson River ships. In addition some empty grain ships at each site were completely sprayed with methoxychlor.

The inspection crews carefully observed the entire grain surface and other sprayed surfaces in each lower hold at each inspection, and after adult moths began to appear, the crews counted the insects in the empty tween deck holds. At the James River Fleet the inspections were made 1 week after the first application, and again 1 week after the second spraying; then 1 week after the third spraying and 1 week before the fourth; and thereafter 1 week after and 1 week before each spraying. At the Hudson River Fleet, inspections were made 1 week after each spraying, and monthly thereafter.

Records were also available from probe samplings of the subsurface areas made routinely by the maintenance crews at each fleet at monthly intervals.

Samples of grain were taken from three holds after each spraying and the residue of pyrethrins was determined by the Chemical Unit of the Stored-Product Insects Section at Savannah, Ga. Samples of the methoxychlor residue were taken by exposing 1-foot squares of sheet aluminum on the walls and floor during the spraying and these were analyzed also.

The empty grain ships sprayed with methoxychlor were inspected once each following the treatment.

RESULTS.—At the James River Fleet only moth larvae were found until near the end of April when a few adults began to appear. No beetles were found until July when an occasional dead one was found and one hold contained a live infestation. In August, 21 of 300 holds contained live beetles, in September 107 of 295 holds, in October 123 of 295 holds, and in November 42 of 295 holds. Psocids also began to appear in August and the number of holds in which psocids were found increased from 12 in August to 122, 150, and 46 in September, October and November, respectively.

At the Hudson River Fleet, only Indian-meal moths were found in the ships, and these were very few in number. During May and June those found were all in the larval stage, and in July they were mostly in the pupal

Table 1.—Insects per hold found in the ships of two fleets in 1957.

Month	James River Fleet					Hudson River Fleet				
	Holds Examined	Moths		Beetles		Holds Examined	Moths		Beetles	
		Alive	Dead	Alive	Dead		Alive	Dead	Alive	Dead
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
April	475	42.2	137.8	0	0	410	0	0	0	0
May	440	7.4	1,380.0	0	0	410	.4	0	0	0
June	355	4.5	2,054.9	0	0	410	0	0	.1	0
July	330	2.4	2,782.0	.8	1.8	365	.02	0	0	0
August	300	24.6	4,180.6	3.4	.9	350	.28	0	0	0
September	295	193.4	4,751.6	7.1	6.7	350	.02	0	0	0
October	295	89.6	3,820.9	10.4	19.9	345	.04	0	0	0
November	295	101.0	787.6	.6	13.0	340	0	0	0	0

stage. In August only larvae were found, indicating the beginning of the second generation.

The average number of moths and beetles found per hold at both fleets is presented in table 1.

The residues of pyrethrins were accumulative in the samples taken after each spraying in one of the ships at the James River Fleet. The average residue found was 4.6, 11.1, 15.0, 14.9, and 34.3, p.p.m. of pyrethrins, respectively, after the first, second, third, fourth, and fifth sprayings.

The average residues of methoxychlor found on the exposed plates 1 week after spraying were 141.5 and 16.4 p.p.m., respectively, for the walls and deck of the tween deck hold. Plates exposed for 6 weeks after spraying showed 34.5 and 121.3 p.p.m. on the wall and deck panels.

Adult Indian-meal moths alighting on methoxychlor-treated surfaces were killed about as rapidly as those contacting the pyrethrum residues. In 9 empty ships completely sprayed with methoxychlor 152 live and 6,737 dead moths were counted. In one of these ships 50 live and 150 dead Angoumois grain moths (*Sitotroga cerealella* (Oliv.), 150 dead flour beetles, 116 live and 600 dead weevils, and 25 live and 75 dead lesser grain borers (*Rhyzopertha dominica* (F.)) were counted.

EVALUATION OF TREATMENTS.—The success of the spraying schedule followed at the Hudson River Fleet was outstanding. In previous years general infestations had developed in many holds to the extent that all holds had been routinely fumigated at least once during the season. The greatest number of insects found in the fleet in any month was 98 live Indian-meal moths and 4 cadelles, *Tenebroides mauritanicus* (L.). Considering that there were 350 or more holds of wheat (table 1), this was a remarkable record of suppression.

At the James River Fleet the sprays successfully suppressed both moth- and beetle-infestations as long as the periodical applications were continued, but when the sprays were discontinued, except on a very few ships, infestations began to occur in numbers. Table 1 shows that the moth populations were at a very low level through July but that an upswing began in August after the spraying was terminated and increased during the remainder of the season. The populations of beetles began to increase at the same time.

The degree of suppression at the James River Fleet was notable in view of the favorable conditions for moth development in that area, and the past history of infestations on these ships. Only 3,278, 1,610, and 803 live moths were present in the whole fleet of 66 or more ships in May, June, and July. This is a very effective demonstration of insect control.

The high degree of effectiveness of the pyrethrum sprays prevented the buildup of moth populations to the point where larval migrations into the empty tween deck holds did not occur as they had in past seasons, so there was no opportunity to evaluate the effectiveness of the methoxychlor residual spray against larvae. As noted, adults alighting on methoxychlor-treated surfaces were killed. The insect counts in the empty ships substantiated these observations.

REFERENCE CITED

Phillips, G. L. 1955. Grain fumigation. Agric. Chemicals, 10(1): 55–6, 117–21.

The Effects of Multiple Gravity on the Life Cycle of an Insect[1,2]

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ABSTRACT

An insect may be one of the most useful and convenient organisms for use in space biology. When the vinegar fly *Drosophila melanogaster* Meigen was reared through two generations in a centrifuge where it was subjected to a continuous force of 10 times gravity (10 G), the time required to develop from egg to adult was only slightly longer than at normal earth gravitation, but there was a reduced yield of insects.

Insects meet several requirements for use as biological indicators in outer-space studies. They stand decompression; they are light in weight, compact, and hardy; and most species have several generations a year. Different

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[2] Appreciation is expressed for advice given by Frank H. Babers, Quartermaster Research & Development Center, U. S. Army, Natick, Mass., and Sarah B. Pipkin, Howard University, Washington, D. C.

species vary in their biology and ecology adaptations. To find out whether an insect can maintain itself under the pressure of multiple gravity, insects were reared in a centrifuge where they were subjected to a continuous force of 10 times gravity (10 G). 10 G also approximates a space vehicle's launching acceleration and the point at which man blacks out (12 G).

The vinegar fly *Drosophila melanogaster* Meigen (Canton wild strain) was selected as the test insect, because it is widely used in genetics, has a short life cycle, and it can be easily reared in a test tube.

METHODS.—Eggs of known age were obtained by holding virgin fruit flies for 4 days before they were mated and then placing the females in shell vials that contained wooden spoons on which the food medium had been poured. In a short time the insects laid large quantities of eggs on the spoons. The eggs were counted and then transferred to $5\frac{7}{8}\times1\frac{1}{16}$-inch Pyrex test tubes containing $1\frac{1}{2}$ inches of rearing medium. Cotton plugs were inserted and rubber bands fitted under the lips of the test tubes to act as cushions. Four prepared test tubes were then placed in each of one or two centrifuges (guards removed) used for a test.

During a test the clinical-model centrifuges, regulated with Variacs, were operated continuously at 240 r.p.m. (fig. 1). This produced a force equivalent to 10 times gravity at a radius of 6 inches as measured to the top of the rearing medium in the test tubes. The formula for computing the force was:

$$\text{Force} = \frac{4\pi^2 r\ (\text{feet}) \left(\frac{\text{r.p.m.}}{60\ \text{seconds}}\right)^2 \times G}{32.2}$$

The r.p.m.'s were checked periodically with a Strobtac. Observations on insect development were made without stopping the equipment by use of a Stroblume synchronized with the centrifuge by a rotor arm and a stationary contact.

In the first experiment 100 eggs were placed in each of four test tubes containing a corn meal mixture. The ingredients for a batch were $\frac{1}{2}$ cup of cornmeal, 6 grams of agar in 600 cc. of water, $\frac{1}{4}$ cup of oatmeal, 10 grams of dried Brewer's yeast, 120 cc. of Karo syrup, 7 cc. of furfural solution, and 8 grams of 95% alcohol.

In the second experiment the number of eggs was reduced to 50 in each of 12 test tubes and a banana-agar medium was used because the corn meal food dried out too rapidly. Two liters of the banana-agar food contained 30 grams of dried yeast, 1,250 cc. of water, 40 grams of agar, 6 bananas, 250 cc. of Karo syrup, and 2 cc. of Moldex (a mold inhibitor).

FIG. 1.—Observations on the development of *Drosophila melanogaster* were made at frequent intervals while the culture was continuously centrifuged at 10 G.

Table 1.—Time table for the development of *Drosophila melanogaster* from egg to adult at normal gravity and at 10 times normal gravity, when reared on different media.

Days from Egg Stage	Reared on Corn-Meal Medium		Reared on Banana-Agar Medium	
	At 10 G	At Gravity	At 10 G	At Gravity
	Number of Pupae			
5	0	4	0	0
6	20	26	16	91
7	62	100	77	364
8	90	272	174	507
9	124	343	212	550
10	—	—	243	568
	Number of Adults			
9	2	18	—	—
10	21	61	—	—
11	48	161	4	106
12	62	241	103	425
13	62	253	131	564
14	66	291	279	590
15	110	324	287	606
16	118	325	—	—
17	123	337	—	—
18	125	347	—	—

RESULTS.—The developmental times for fruit flies reared in the cornmeal and banana-agar media at 10 G and normal gravity are given in table 1.

There was total loss of adults through their becoming mired and drowning in the food before they were able to lay eggs for the F_2 generation. In an attempt to prevent this loss, excelsior was loosely packed in the empty area above the food to afford a resting place for the adults as they emerged from the pupal cases on the side walls of the tubes. Otherwise the conditions were the same as in the second experiment except that eight tubes were used for the experimental group and the same number for the controls. The results of this experiment are given in table 2.

Table 2.—Maintenance of *Drosophila melanogaster* at 10 G over a 35-day period by the use of excelsior to provide adult resting areas.

Rearing Tube No.	Number of Pupae+Pupal Cases	Number of Live Adults	Number of Larvae
	F_1 Generation—17 Days from Egg Stage[a]		
1	25	1 male	0
2	33	1 female	0
3	25	1 female	0
4	29	2 males	0
Controls	28 (av.)	27 (av.)	Many
	F_2 Generation—35 Days from Egg Stage		
5	81	12[b]	Many
6	27	0	0
7	62	10[c]	Many
8	18	0	0
Controls	>100	75 (av.)	Many

[a] Centrifuge permanently stopped on 17th day.
[b] 22 after 20 minutes.
[c] 20 after 20 minutes

At 70° to 80° F., when kept in a continuously lighted room (fluorescent lights), the vinegar flies became adults in 9 to 18 days after the 1-day egg stage. Insects reared at 10 times gravity became adults almost as soon as those reared at normal gravity. Of 1,000 eggs in each group, 412 reached the adult stage when exposed continuously at 10 G while 953 completed the life cycle in the controls. Banana-agar food at a density of 50 eggs per tube was superior to corn meal at 100 eggs per tube.

By placing excelsior in the tubes it was possible to rear vinegar flies through two generations at 10 G. The centrifuges stopped for 1 minute on the sixth day. Larvae, probably of the third generation, were present when the experiment was concluded on the 35th day.

From these results it appears that the vinegar fly is highly suitable for use in space biology under conditions where temperature and the oxygen-carbon dioxide balance can be maintained.

Laboratory Rearing of a Pine Cone Insect, *Dioryctria abietella* (D. & S.)[1]

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ABSTRACT

A satisfactory method for continuous rearing of *Dioryctria abietella* (D. & S.) has been developed. Larvae are maintained in petri dishes using paraffin-waxed, first-year pine cones as food. The duration of the life cycle was slightly over 1 month under summer conditions. Survival to the adult stage has ranged from 50% upward. In a 1-year period about 1,000 moths were reared, and 1,600 larvae developed to late instars before use in insecticide tests.

In the vicinity of Olustee, Florida, during the summer of 1957, frequent collections were made of second-year slash pine cones that had obviously been damaged by insects. Most of the damage was being caused by the larvae of two species of cone moths, *Dioryctria abietella* (D. & S.) and *D. amatella* (Hulst).

It was desirable to establish and maintain colonies of these moths in the insectary and laboratory to provide larvae both for biological studies and for insecticide screening tests. In Canada, Lyons (1957) obtained unfertilized eggs from adult *D. abietella* reared from red pine cones; however, no method of maintaining *Dioryctria* cone moths in continuous culture has been recorded. A satisfactory technique for rearing *D. abietella* has been developed at the Lake City Research Center and is described in this paper. The same technique has also been used to rear *D. amatella* from egg to adult, but further work is needed to maintain this insect in continuous culture.

METHODS—*Obtaining and Handling of Moths.*—The

[1] Accepted for publication December 19, 1958.

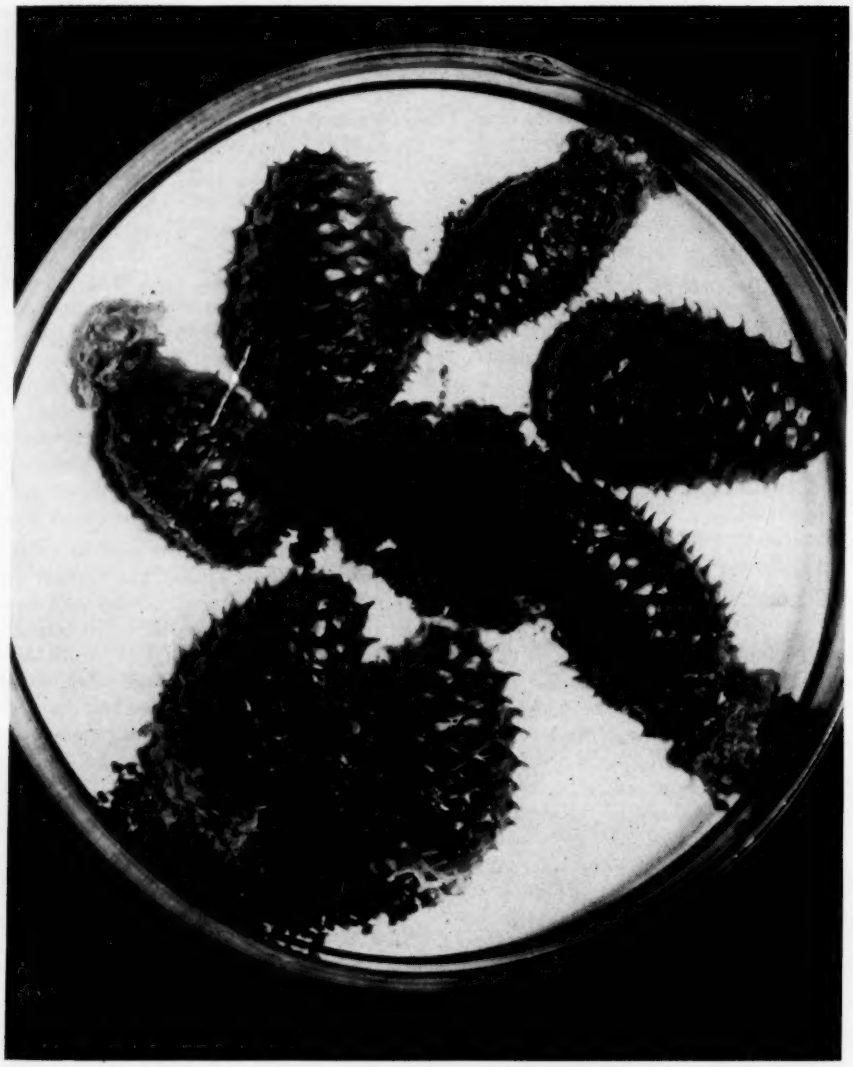

FIG. 1.—Technique of rearing *Dioryctria abietella* in petri dish. Larvae were provided waxed conelets until they matured. Accumulation of pellets is larval frass.

original moths were obtained from field-collected cones held in various screened cages and lard cans in the insectary. The majority of subsequent adults have been reared from stock cultures in petri dishes (fig. 1) from late summer of 1957 through July 1958. An additional group of wild moths from rust-infected, first-year slash pine cones was placed in culture during the spring of 1958. Progeny of these were gradually combined with over-wintered laboratory cultures to prevent possible ill effects of continuous inbreeding.

Newly emerged moths were caught in small shell vials and released to breed in screened cages approximately 1-foot square. Vials of sugar water were included to provide food and moisture. Unfed moths usually have not survived or have not laid fertile eggs.

Females were isolated after remaining in the breeding cage for 2 days. They were placed in half-pint, wide-mouth Mason jars, usually two moths per jar. The jar lid was replaced with a circle of 32-mesh saran fabric or with cheesecloth. A 9.0-cm. filter paper disk was folded to fit the bottom and one side of the jar and moistened, or a small vial of sugar water was provided to supply moisture to the moths. A strip of cheesecloth about 1½-inch wide and 3 inches long pleated several times, was placed with the moths for egg deposition. The moth isolation setup is shown in figure 2.

Egg-laying usually started shortly after isolation and continued until the moth died. Females lived for several days to several weeks. About 75% of the females laid fertile eggs when approximately equal numbers of males and females were present in the breeding cages before isolations were made.

Handling of Eggs.—Fertile eggs can be readily identified by a color change from pale yellow to bright orange within the first day of development. The eggs deposited on cheesecloth strips (fig. 3) were removed as soon as coloring was noticed and placed on moist filter paper in covered petri dishes to hatch. Hatching normally occurred

within 3 to 7 days after egg-laying, apparently depending upon temperature. Ordinarily, most of the fertile eggs hatched successfully.

Larval Handling and Care.—When the eggs were nearly ready to hatch, the egg shell was distinct from the embryo, and the small larva could readily be seen through the shell. Two methods of handling the hatching larvae may be used. If time was available to remove the larvae as they hatched they were transferred directly to permanent food. Otherwise, temporary food was supplied to the larvae in the hatching dish. Individual scales from first-year cones of longleaf or slash pine are excellent temporary food. Larvae, usually feeding externally on these scales, were easily removed. Such scales remained suitable for several days on the moist filter paper and could be replenished if necessary.

For the continued rearing of the larvae, more permanent food material is necessary, and first-year longleaf or slash pine cones were very satisfactory for this purpose. These were used intact during early summer, or halved longitudinally as conelets became larger. They were dipped in melted paraffin to delay desiccation both in storage prior to use and while in use as larval food. Such conelets or conelet halves were usable after as long as 2 months in refrigeration. When used, the stem ends were cut off to provide a point of entry for young larvae. Four such cones were usually placed on moistened filter paper in covered petri dishes. A single larva was then transferred with a moistened camel-hair brush from the hatching dish to each conelet. The larvae usually fed singly in individual conelet halves.

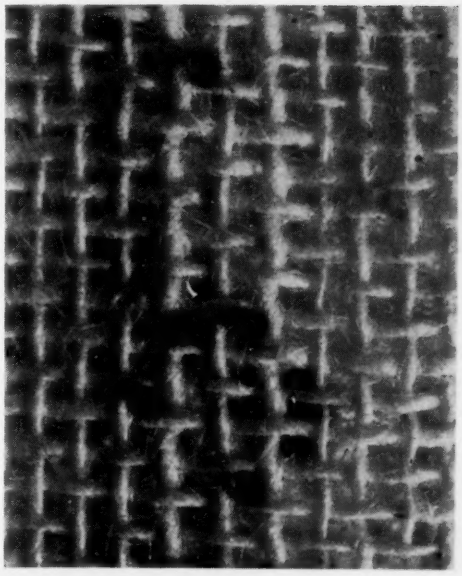

FIG. 3.—*Dioryctria abietella* eggs deposited on cheesecloth strips. Pigmented areas on the eggs indicate normal development.

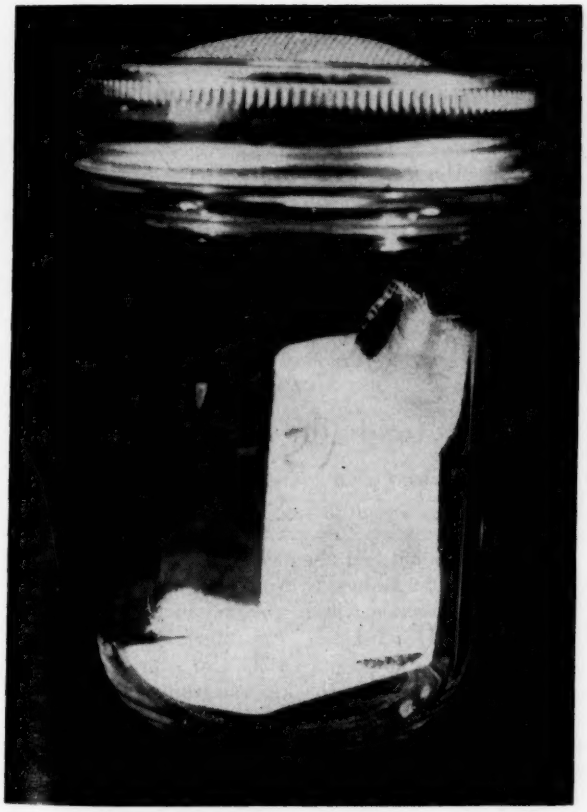

FIG. 2.—Female *Dioryctria abietella* moths ovipositing on pleated cheesecloth strip in an isolation jar.

During periods of dry weather, filter papers were moistened when the larvae were set up and at about weekly intervals thereafter. Sufficient moisture should be present to prevent the conelets from drying out. Excessive moisture, however, particularly during humid weather, promoted on the cones a fungus growth which in some instances appeared to hamper larval development or cause mortality, possibly by rendering the conelets unsuitable for food.

Larvae in culture were refed once with fresh waxed conelets; when the old conelets were found to be nearly consumed, the new ones were placed beside the old ones in which larvae were feeding. Larvae ate nearly all of each conelet, leaving only an outer shell of cone tissue and the wax coating. The larval period was completed in about 17 days under summer conditions.

Pupation and Moth Emergence.—Larvae usually pupated either under a cone half or on the filter paper in a thin frass-covered cocoon. The duration of the pupal stage was about 11 days during the summer.

When most of a group of larvae had pupated, the filter papers and conelets with attached cocoons were transferred to screened cages. Moths emerging in these cages were removed daily and placed in a breeding cage to mate and continue the colony.

RESULTS.—During 1958, large-lot survival of larvae to the adult stage when reared by the technique outlined above ranged from 54 to 75% (table 1).

During the year in which rearing has been carried on about 1,300 moths have been used to provide larvae for establishing and continuing the colony in the insectary and laboratory and to provide larvae for insecticide screening tests. About 200 of these moths were obtained from field-collected cones during the summer of 1957 to

establish the colony. An additional 100 moths from rust-infected cones were added to the colony during the spring of 1958. The remaining 1,000 moths used were reared by the technique described in this paper. In addition, about 1,600 larvae have been reared to late instars and used for insecticide screening tests during the spring and early summer of 1958.

Table 1.—Survival of larvae[a] of *Dioryctria abietella* in rearing at Olustee, Florida, 1958.

Months	Number of Larvae	Number of Adults	Per Cent Survival
May–June	300[b]	164	55
June–July	268[b]	144	54
June–July	300[c]	224	75

[a] First- and second-instar larvae transferred to waxed cone halves. The first instar is transient; hence part of the population changes to the second instar in the hatching dishes.
[b] Obtained from overwintered stock colony.
[c] Obtained from adults reared from 1958 rust-infected cones.

DISCUSSION.—Currently 300 larvae are established as a stock colony for each generation reared. Such a colony then provides larvae for insecticide tests and for perpetuating the colony, as well as allowing a margin of safety in case of adverse rearing conditions. Rearing time from egg to adult under summer conditions or in a regularly heated building requires slightly over 1 month but is greatly extended by cool weather.

As the rearing technique has been developed, several problems have arisen. The most severe of these was the apparent effect of high temperature on the adult moths. During July 1958, maximum air temperatures recorded in the insectary ranged from 88° to 100° F., averaging 94.7° F. About 200 moths which emerged and were transferred to breeding cages under these conditions died before females were isolated. Removal of the cages to an air-conditioned room increased moth survival, but relatively few females lived past a single day of isolation, and egg-laying was much below an approximate normal of 50 eggs per female obtained in prior rearings. It seems probable that effects of high temperatures on larvae or pupae may also be reflected by reduced vigor and fecundity of adults.

While facilities were not available to ascertain optimum temperatures for rearings, they were very successful when maximum temperatures did not exceed 85° F., and usually ranged from about 60° to 80°. Winter night temperatures as low as 20° F. did not injure adult moths; however, failure of several groups of eggs to develop may have been caused by alternating heat and cold in an irregularly heated laboratory room. Other egg lots held in the insectary during periods of extended cool weather hatched irregularly over a 2- to 3-week period, while similar eggs in the heated laboratory hatched within a week of deposition.

Several other difficulties should also be reported. Sugar-water vials provided for the adult moths should have the feeding plug flush with the top of the vial. If it protrudes, it may either dry out rapidly or cause the vial to drip and damage the moths. Several species of ants have proven to be serious pests, particularly in breeding cages, where they have attacked moths, and in egg-holding dishes, where they fed on the eggs. Setting the cages and dishes on inverted metal boxes or similar objects ringed with a 1-inch band of tanglefoot has prevented ant invasion. Larval transfer, where larvae are placed directly on the cut stem-end of a waxed conelet, has resulted in entanglement and death of some larvae in the exuding resin. Larvae seemed to survive better when placed on the waxed cone surface and allowed to find their own way to the cut cone end or to a break in the wax coating.

REFERENCE CITED

Lyons, L. A. 1957. Insects affecting seed production in red pine II. *Dioryctria disclusa* Heinrich, *D. abietella* (D. & S.), and *D. cambiicola* (Dyar) (Lepidoptera: Phycitidae). Canadian Ent. 89(2): 70–79.

Field Evaluation of Thiodan as an Insecticide for Potatoes[1]

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ABSTRACT

Over a period of 3 years field tests were conducted to determine the effectiveness of Thiodan®, identified chemically as 6,7,8,9,10,-10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide, against foilage infesting insects on potatoes. Results of these tests demonstrated that this material gave excellent control of aphids, potato flea beetle (*Epitrix cucumeris* (Harr.)), potato leafhopperm, (*Epoasca fabae* (Harr.)), and Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) on both an early variety and a late variety of potatoes. Thiodan was found to be compatible with such fungicides as Dithane (nabam), copper oxychloride sulfate (COCS), and Bordeaux mixture.

Thiodan®, identified chemically as 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide was discovered in the laboratories of Farbwerke Hoechst A.G., Frankfurt, Germany. It was brought to this country in 1955 and shortly thereafter the Niagara Chemical Division was granted the exclusive right to develop the compound within the United States. In the short time since introduction, enough investigators have published on their studies to show that Thiodan is effective against a relatively broad spectrum of insect

[1] Accepted for publication December 22, 1959.

species. Lindquist & Dahm (1957) were among the first to investigate and report on the synthesis of Thiodan. At the same time they noted that the acute oral toxicity to rats was approximately 50 mg./kg. of body weight. The compound was judged to be slightly more toxic than DDT when applied topically to a normal strain of house flies. Work by Allen, *et al.* (1957) demonstrated the effectiveness of Thiodan for the control of the cyclamen mite, *Steneotarsonemus pallidus* (Banks). Ditman *et al.* (1958) also noted that Thiodan was very promising for the control of the cabbage looper, *Trichoplusia ni* (Hbn.), the imported cabbage worm, *Pieris rapae* (L.) and the cabbage aphid, *Brevicoryne brassicae* (L.). Ditman & Wiley (1958) in reporting the effectiveness of several insecticides for the control of insects on snap beans noted that Thiodan was effective in controlling the Mexican bean beetle, *Epilachna varivestis* Muls. and the potato leafhopper, *Empoasca fabae* (Harr.). Hofmaster (1958) presented data to show that the compound was effective against the potato leafhopper. At the same time he reported good control of the potato flea beetle, *Epitrix cucumeris* (Harr.) and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Landis (1958) reported that Thiodan gave good control of aphids, leafhoppers, and potato flea beetles as well as other insects and mites on potatoes.

Several popular release references (Anon. 1958a,b,c.) point to successful control of catfacing insects of the stink bug type on peaches and the green peach aphid, *Myzus persicae* ((Sulz.) on potatoes. Of interest and of extreme importance was reference to the fact (1958a) that large scale field applications of Thiodan to alfalfa did not interfere with the honey bee populations.

Fulton & Smith (1958) list several respirators and gas mask canisters suitable for use when working with this material. It is the purpose of this paper to present results collected over a period of 3 years of experiments examining Thiodan for the control of potato foliage insects.

METHODS AND MATERIALS.—All tests were conducted at the Niagara Research Farm, Middleport, N. Y. All compounds were applied with a Hardie Sprayer operated at about 150 p.s.i. The sprayer was equipped with a 9-foot boom on which were installed eight cone nozzles. The machine traveled at a rate to deliver 100 to 125 gallons of dilute spray per acre. Plots consisted of 20 foot segments of the two outside rows of a five row planting. This allowed four nozzles per row. An entire row between plots was left untreated in addition to the several replicated untreated plots that were a part of the experiment. Treatments comprised four to six replicates and applications were made at 7- to 10-day intervals.

Table 1.—Effectiveness of Thiodan for the control of potato leafhopper and Colorado potato beetle.

MATERIAL[1]	POUNDS ACTIVE PER 100 GALLONS	*E. fabae* NYMPHS PER 100 LEAVES		COLORADO POTATO BEETLE LARVAE PER PLOT
		July 28	Aug. 9	Aug. 9
Thiodan 25% (WP)	0.5	24	24	0
Thiodan 25% (WP)	.75	4	0	0
Thiodan (EC) 2	.5	0	0	1
Parathion 15% (WP)	.3	0	98	0
Untreated	—	340	186	32

[1]Applied July 26, 1956.

Counts were made on entire leaves when the insect population was heavy enough to determine any differences in control. Potato leafhopper nymphs, aphids, and potato flea beetle punctures were counted on 10 to 50 entire leaves from each of the replicates. Counts of the Colorado potato beetle were made as the total number of larvae found in each of the six replicates per treatment. In general there were six replicates of each treatment. The condition of the foliage was determined by two individuals estimating the per cent foliage alive in all replicates and averaging these estimates. Yields were obtained by weighing the potatoes found in all of the replicates of each treatment.

Early Irish Cobbler plots in 1956 and 1957 received no fungicide application. The 1958 Irish Cobbler potatoes received one application of copper oxychloride sulfate (COCS) at 3.0 pounds per 100 gallons. In a compatibility study on late-season potatoes, the Green Mountain variety, Thiodan was used in combination with each of several fungicides. These included COCS, Bordeaux mixture and Dithane D-14 (nabam). The COCS was used in a manner to guarantee 2 pounds of copper expressed as metallic. The Bordeaux mixture was prepared from 8 pounds of $CuSO_4$ and 4.0 pounds of hydrated lime, all diluted to 100 gallons. Dithane D-14 (22%) was used at the 2-quart rate and to it was added 0.75 pound of zinc sulfate per 100 gallons. Thiodan at a rate of 2 pounds of 25 per cent wettable powder was used with each of these.

RESULTS.—Table 1 shows the results obtained in 1956 from a single application of Thiodan for control of the

Table 2.—Control of foliage insects on potatoes with all-season applications of Thiodan. 1957.

MATERIAL	POUNDS ACTIVE PER 100 GALLONS	FLEA BEETLE PUNCTURES PER LEAF ON		*E. fabae* NYMPHS PER LEAF JULY 22	POTATO APHIDS PER LEAF JULY 22	YIELD (BU./A.)
		June 17	July 22			
Dieldrin (EC) 15%	0.18	2.6	4.1	5.8	1.3	306
Thiodan 25% (WP)	.25	1.1	1.6	2.5	0.3	302
Thiodan 25% (WP)	.5	0.9	2.2	0.5	0.4	334
Thiodan (EC) 2 lb./gal.	.5	1.6	1.1	0.2	0.3	340
Parathion (EC) 2 lb./gal.	.25	2.7	16.1	4.8	1.4	328
Untreated	—	11.9	21.9	9.8	1.1	299
L.S.D.—Among chemical treatments at 1% level[a]	—	1.65	5.4	3.12	0.96	—

[a] The cooperation of Dr. K. P. Dorschner in the determination of the statistical analysis is gratefully acknowledged.

Table 3.—Compatibility of Thiodan with various fungicides when used to control insects on Green Mountain potato foliage. 1957.

Material and Rate per 100 Gal.	Number of Flea Beetle Punctures/Leaf		Colorado Potato Beetle Larvae/ Plot	Aphids/ 50 Sweeps	*E. fabae* Nymphs/ 50 Sweeps	Per Cent Leaves Injured by *E. fabae*	Per Cent Foliage Alive on		Tubers with Blight	Yields
	June 19[a]	Aug. 1[b]	July 8	July 12	July 12	Aug. 1	Sept 23	Oct. 9	(%)	(Bu./ A.)
Thiodan 25% (WP) 2.0 lb. +COCS 3.6 lb.	2.2	5.9	0	46	49	13	95.1	71.9	2.1	549
Thiodan 25% (WP) 2.0 lb. +copper sulfate 8.0 lb. +lime 4.0 lb.	1.7	5.1	0	56	43	13	90.0	60.0	4.3	542
Thiodan 25% (WP) 2.0 lb. +Dithane D-14 2.0 qts. +zinc sulfate 0.75 lb.	1.1	3.8	0	21	44	13	93.9	78.1	6.2	582
Thiodan 25% (WP) 2.0 lb.	1.2	5.2	0	40	27	13	80.2	27.5	37.7	536
Untreated	31.6	37.1	31.5	205	196	80	31.2	1.5	55.5	391

[a] First brood.
[b] Second brood.

Colorado potato beetle and potato leafhopper nymphs. Counts made 48 hours after the treatment showed a large (70 to 100%) reduction of potato leafhopper nymphs. Counts made 2 weeks after the application showed that Thiodan either as a wettable powder or as an emulsifiable concentrate was more effective than the standard parathion for control of potato leafhopper nymphs and as effective as the standard against the Colorado potato beetle.

Results of the 1957 (all-season applications on Irish Cobbler potatoes) field trials are found in tables 2 and 3. Counts of potato flea beetle punctures made on June 17 and on July 22 showed that Thiodan had been more effective than parathion or dieldrin. Counts of potato leafhoppers on July 22 showed that Thiodan was more effective than either of the standard materials. (Table 2).

Thiodan at rates of 0.5 pound of active ingredient per 100 gallons either as a wettable powder or as an emulsifiable concentrate increased the yield over the untreated plots and to a lesser degree increased the yield over the parathion standard.

Field trials on late Green Mountain potatoes in 1957 are reported in table 3. This test was set up primarily for a compatibility study of Thiodan with currently recommended fungicides. Counts of punctures from the first brood of potato flea beetles on June 19, and from the second brood on August 1, showed that Thiodan gave good control either alone or in combination with any of the fungicides. Counts of the Colorado potato beetle larvae showed 100% control in all Thiodan plots. On August 1 an examination of the foliage injured by potato leafhopper showed that plots treated with Thiodan alone or in combination with any of the fungicides had considerably less injury than the untreated plots.

The per cent of foliage alive was estimated on September 23 and again on October 9. Plots treated with Thiodan plus any of the fungicides had more live foliage than plots treated with Thiodan alone, or untreated check plots showing that Thiodan did not inhibit the activity of the fungicide. The great difference between the amount of foliage alive on the untreated check and on the plot treated with Thiodan alone was due mostly to injury by

Table 4.—Control of insects on Irish cobbler potato foliage with Thiodan. 1958.

Material[b]	Pounds Active/100 Gal.	Potato Aphids/ Leaf June 25	Aphids[a]/ Leaf July 8	Flea Beetle Punctures/Leaf on June 25	Flea Beetle Punctures/Leaf on July 8	*E. fabae* per Leaf July 15	Yield (Bu./A.)
Thiodan 25% (WP)	0.25	2.6	9.3	0.7	1.4	0.3	184
Thiodan 25% (WP)	.5	0.9	8.7	.3	1.0	.3	213
Thiodan (EC) 2	.25	1.5	4.7	.2	0.7	.2	201
Thiodan (EC) 2	.5	1.0	4.9	.2	0.7	.1	187
Thiodan (EC) 4	.5	0.5	5.1	.5	0.7	.1	196
Thiodan (EC) 2	.75	0.3	3.9	.1	1.1	.1	179
Parathion (EC) 2	.25	0.9	5.5	1.4	1.0	.2	167
Dieldrin (EC) 15%	.187	5.6	26.5	1.5	1.6	.9	176
DDT (EC) 25%	.8	4.5	18.4	1.7	1.3	.02	156
Untreated	—	13.3	26.9	6.7	3.1	1.3	149
L.S.D.—Among chemical treatments 1% level	—	1.43	10.8	1.08	ns	0.45	

[a] Green peach aphid and potato aphid (*Macrosiphum solanifolii* (Ashm.)).
[b] Treated 6/6, 6/16, 6/24, 7/2, 7/9, 7/18, 7/25.
All treatments were replicated six times.

the potato leafhopper. All plots treated with Thiodan alone or in combination with a fungicide increased the yield per acre over that of the untreated.

The 1958 results of tests on early Irish Cobbler potatoes are given in table 4. Thiodan at the rate of 0.5 pound of active material per 100 gallons was as effective as parathion and more effective than the DDT for control of the potato aphids throughout the season. At the same rate it was more effective than the three standards against the potato flea beetle. Counts of the potato leafhopper on July 15 showed that Thiodan was as effective as the standard materials. All Thiodan treatments increased the the yields over any of the standard treatments.

REFERENCES CITED

Allen, William W., H. Nakakihana, and G. H. Schaefers. 1957. The effectiveness of various pesticides against the cyclamen mite on strawberries. Jour. Econ. Ent. **50**(5): 648–52.

Anonymous. 1958a. Pesticides vs. honeybees. Agric. Chemicals **13**(8): 84.

Anonymous. 1958b. New pesticides for orchards. Agric. Chemicals. **13**(9): 27.

Anonymous. 1958c. 32nd Annual Western Spray Conference. Agric. Chem. **13**(2): 57.

Ditman, L. P., George Burkhardt, A. Kramer and H. S. Todd. 1958. Chemical control of insects attacking broccoli and cabbage. Maryland Agric. Expt. Sta. Bull. **A-94.**

Ditman, L. P., and Robert C. Wiley. 1958. The effectiveness of several insecticides for control of insects on snap beans. Jour. Econ. Ent. **51**(2): 258–9.

Fulton, R. A., and F. F. Smith. 1958. Respiratory protective devices. Agric. Chemicals **13**(9)22–24.

Hofmaster, R. N. 1958. The potato leafhopper and its control. Veg. Growers News **12**(12): 3.

Landis, B. J. 1958. How to control aphids, leafhoppers, potato flea beetles, other insects and mites on potatoes. Proc. of the 1958 Potato School, State Coll. of Washington. pp. 57–59.

Lindquist, Donald A., and Paul A. Dahm. 1957. Some chemical and biological experiments with thiodan. Jour. Econ. Ent. **50**(4): 483–6.

Effect of *Pyrausta nubilalis* (Hbn.) on the Growth of Leaves and Internodes of Field Corn[1]

H. C. CHIANG and F. G. HOLDAWAY[2]

ABSTRACT

A 4-year study on the effect of infestation by the European corn borer (*Pyrausta nubilalis* (Hbn.)) on the growth of corn plants shows the following points:

1) Borer infestation adversely affects the elongation of the plants, results in smaller leaves, shorter internodes, and a delay in both tasselling and silking. 2) Reduction in size of leaves increases progressively toward the tip of the plant. 3) The reduction in the length of internodes, however, is greater in internodes near the ear and tassel than in other internodes. 4) The decrease in the length of internodes began when only the leaf blades were injured by the borers, and without the physical destruction or obstruction of the vascular bundles in the stalk. 5) These facts indicate a need for investigations on (a) feeding activities of the young larvae and plant reaction to such feeding; (b) whether a phytotoxic secretion may be involved; and (c) the possibilities of interrelationships between micro-organisms and corn borer infestation.

The normal growth of the internodes and leaves of corn has been studied by botanists. It is known that there are two phases in the growth of the plant structures: 1) the formative period during which the number of structures, such as the leaves and internodes, is determined; and 2) the elongation or enlargement period during which the size of the structures is determined (Martin & Hershey 1935). Anderson (1949) showed that the relative lengths of internodes may be different in different varieties. Sharman (1942) showed that elongation takes place first in the leaf blade, then in the sheath, and last in the internode below. Heimsch & Stafford (1952), who studied the growth of some different varieties, concluded, however, that the elongation of the sheaths of upper leaves and the "subtending" internodes occurred simultaneously.

The growth of leaves and internodes of corn as affected by the infestation of the European corn borer *Pyrausta nubilalis* (Hbn.), however, has not been studied by botanists or entomologists, although a great deal of work has been done on the effect of the corn borer on the growth of the ear and on breakage of stalks and ear shanks.

The present paper deals with comparisons of the size of leaves and the length of internodes of normal and borer-infested corn plants. Some reference is made to the effect of borer infestation on the time of tasselling and silking. Certain aspects of the growth of internodes have been explored in greater detail.

Since the vegetative growth of the plant precedes and influences the growth of the ear, the present study may

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Table 1.—Effect of three levels of European corn borer infestation on the height of plants of two varieties of corn, and of two dates of planting.

Planting and Year	Average Plant Height in Inches				Per Cent Reduction in Height[a]		
	Borer-Free	Infestation			Infestation		
		Light	Medium	Heavy	Light	Medium	Heavy
	MF9×W14 (Susceptible)						
Early							
1953	91.0	90.9	89.1	86.8	0.1	2.1	4.5
4	96.0	93.0	83.0	81.3	3.1	13.5	15.3
5	89.4	88.6	82.3	81.4	0.9	7.9	8.9
6	97.2	87.5	84.7	86.8	10.0	12.8	10.7
7	105.2	99.0	85.5	86.4	5.9	18.7	17.9
Average	95.8	91.8	84.9	84.5	4.2	11.4	11.8
Late							
1953	87.6	86.0	82.8	82.8	1.8	5.5	5.5
4	97.5	96.7	87.1	84.0	0.8	10.7	14.9
5	87.4	87.4	84.2	80.5	0	4.7	7.9
6	89.0	90.2	85.8	83.2	—	—	1.0
7	110.7	107.8	92.5	90.5	2.6	16.4	18.3
Average	93.4	93.6	86.5	84.2	—	7.4	9.8
	Oh43×Oh51A (Resistant)						
Early							
1953	91.3	91.6	91.6	90.9	—	—	0.4
4	94.8	96.7	88.0	88.0	—	7.2	7.2
5	89.8	89.2	84.6	84.1	0.7	5.8	6.3
6	102.0	100.8	103.8	101.5	1.2	—	0.5
7	95.7	95.8	85.5	84.6	—	10.7	11.6
Average	94.7	94.8	90.7	89.8	—	4.2	5.2
Late							
1953	88.8	90.4	88.7	88.3	—	0.2	0.6
4	92.2	92.3	90.6	92.1	—	1.8	0.1
5	89.9	89.8	89.8	86.6	0.2	0.2	3.7
6	92.7	94.2	94.0	92.5	—	—	0.3
7	102.8	103.3	95.1	93.7	—	7.6	8.9
Average	93.3	94.0	91.6	90.6	—	1.8	2.9

[a] The height of borer-free plants was used as the check in computing the per cent reduction in height due to borer infestation. The check plants were sprayed with EPN, which may have had some effect on plant growth. Since the heights of plants with different levels of borer infestation are compared with the same check in a given year and a given planting, the possible effect of EPN, if any, would not alter the trend of the results.

pave the way for a more critical evaluation of the effect of borer infestation on corn yield.

MATERIAL AND METHODS.—The study was carried out at the Southern Experiment Station, University of Minnesota, at Waseca, Minnesota, during 1954 to 1957. Two single-crosses were planted each at two different times during the period of planting normal for southern Minnesota. The two lines were WF9×M14, which is rated susceptible on the basis of its reaction to borer infestation, and Oh43×Oh51A, which is resistant. Most of the detailed studies were on the susceptible cross only.

In order to study the effect of borer infestation, plants that were borer-free were compared with plants with three different levels of borer infestation as follows:

1. *Check plots, borer-free.* Plants were sprayed repeatedly with wettable EPN at a rate equivalent to one-half pound of active insecticide per acre during the period of first-brood oviposition and hatching of the eggs.

2. *Light infestation plots.* Plants were exposed to oviposition by the field populations of the first-brood moths. The natural infestation was light during the entire period of the study.

3. *Medium infestation plots.* Plants were exposed to natural first-brood infestation and were also infested artificially with three first-brood egg masses per plant.

4. *Heavy infestation plots.* Plants were exposed to natural first-brood infestation and were also infested artificially with six first-brood egg masses per plant.

The egg masses used for artificial infestation were produced in the laboratory and were placed in the whorl of the plant. They were placed on the plant at a time when the stage of plant growth was favorable for borer survival and also when natural oviposition was taking place.

Records on the total height of plants, the length of internodes, and the size of leaves were taken, in most cases, when the plants reached their maximum growth and, in some cases, at intervals during the season. Records of the injury sites and the numbers of borers on the plants were also taken for some special purposes.

The present study was carried out concurrently and in conjunction with a north-central regional study on corn borer populations (Regional Project NC-20). Thus the varieties, dates of planting, and methods of infestation, spraying and checking of borer infestation were the same as for the regional project. For details of the procedures, readers are referred to Everett *et al.* (1958).

RESULTS: 1. *Plant Height in Relation to Level of Borer Infestation and the Variety of Corn.*—The maximum height of plants in the four plantings and in plots of the four levels of borer infestation are given in table 1.[3] These data show the following points:

a) The reduction in the height of plant was proportional to the severity of borer infestation. This was particularly clear in the susceptible single-cross, WF9×M14.

b) Within the same variety and the same level of in-

[3] These records were taken in the NC-20 experimental plots and are published here with the approval of the NC-20 Technical Committee

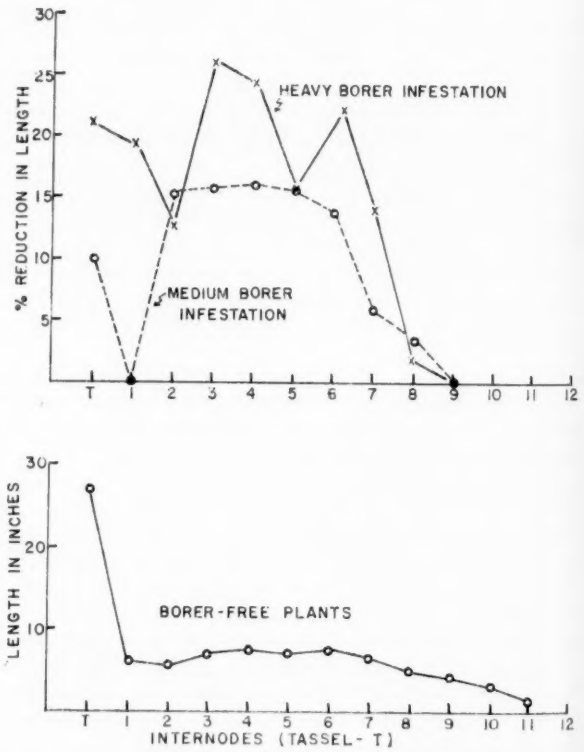

FIG. 1.—Upper, per cent reduction in the length of internodes of plants with two levels of borer infestation. Lower, length of internodes of borer-free plants. "T" is tassel, and the internodes are numbered from the tassel down. Early planting WF9×M14, 1954 records.

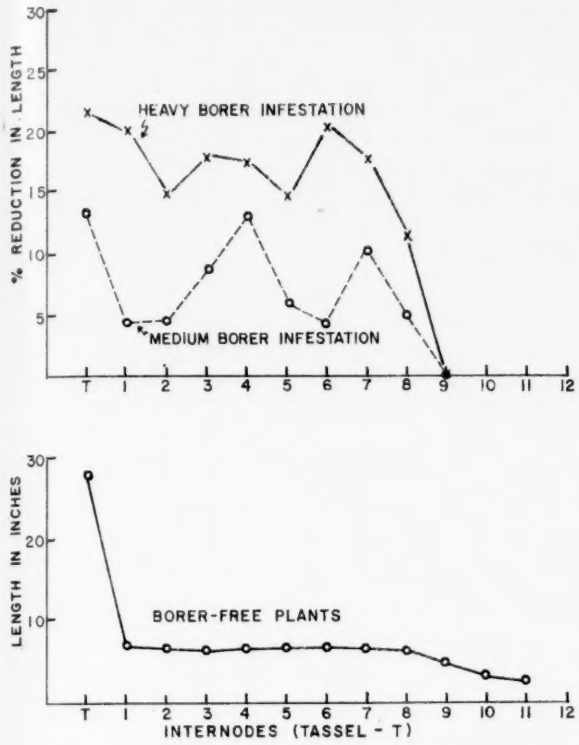

FIG. 2.—Upper, per cent reduction in the length of internodes of plants with two levels of borer infestations. Lower, length of internodes of borer-free plants. Late planting WF9×M14, 1954 records.

festation, in general, the early planting showed a greater reduction than the late planting.

c) Within the same planting date and same level of infestation, the susceptible single cross suffered a greater reduction than the resistant single cross.

d) The reduction in plant height in a given planting and with a given level of borer infestation varied also with the season.

2. *The Internodes Where Reduction in Length Takes Place.*—The information given above leads to the question, which internodes are responsible for the reduction in the over-all height of the plant? In order to clarify this point, the lengths of individual internodes were measured in 1954. Since the susceptible single cross showed the greatest reduction caused by borer infestation, detailed records were taken only on this cross, and are given in figures 1 and 2.

The actual length of internodes of the borer-free plants shows a gradual increase toward the top of the plant; the tassel was several times longer than any of the internodes. The former fact confirms the findings of Anderson (1949). The reduction of the various internodes shows two consistent characteristics: 1) the basal three internodes were not affected at all. This may be explained by the fact that these internodes were completely extended when the borer infestation began; 2) as in the reduction of the overall plant height, the reduction in the length of individual internodes was also greater with heavy infestation than with medium infestation.

The higher internodes, however, showed varying degrees of reduction as indicated by the zigzag trend of the curves. In order to determine if the irregularity was due to sampling errors, the observations were repeated in 1957. The results are given in figures 3 and 4. The pattern of the lengths of the internodes and tassel of borer-free plants that was shown in the 1954 records was again shown in the 1957 records. Furthermore, the peculiar pattern of the per cent reduction in the lengths of the higher internodes owing to borer infestation was also reproduced.

Since it appeared that this peculiar pattern was more than a coincidence and was not correlated with the normal growth of the internodes, possible explanations were sought in 1957 along the line of borer injury on the plants. The distribution of sheath damage and tunnels in the internodes was checked. Ten plants were examined, after they reached their maximum height, in the heavy infestation plots of the early as well as the late planted susceptible WF9×M14. The percentage of plants showing injury on a given sheath or internode is shown in the lower graphs in figures 3 and 4. The results show that the injury was more abundant in plants with heavy infestation than in those with median infestation. The results also show

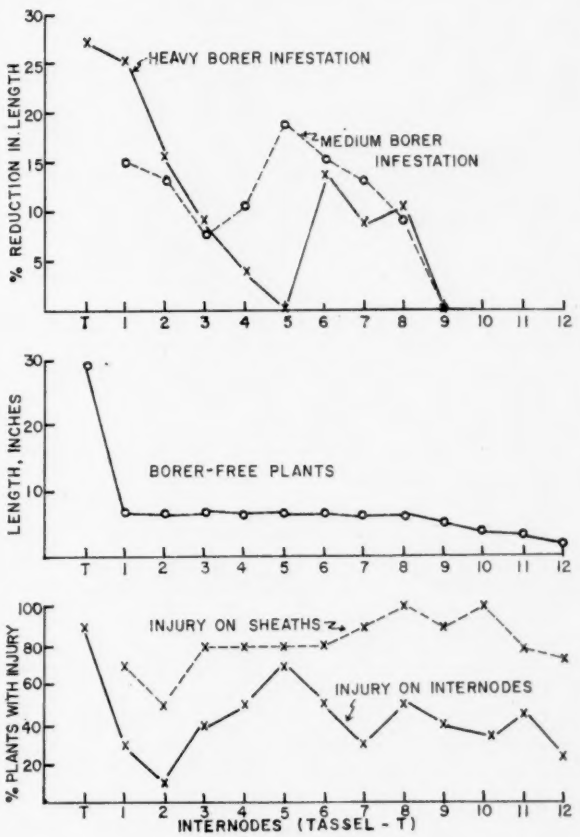

FIG. 3.—Upper, per cent reduction in length of internodes of plants with two levels of borer infestation. Middle, length of internodes of borer-free plants. Lower, injury on internodes and on leaf sheaths on plants with heavy borer infestation. Early planting WF9×M14, 1957 records.

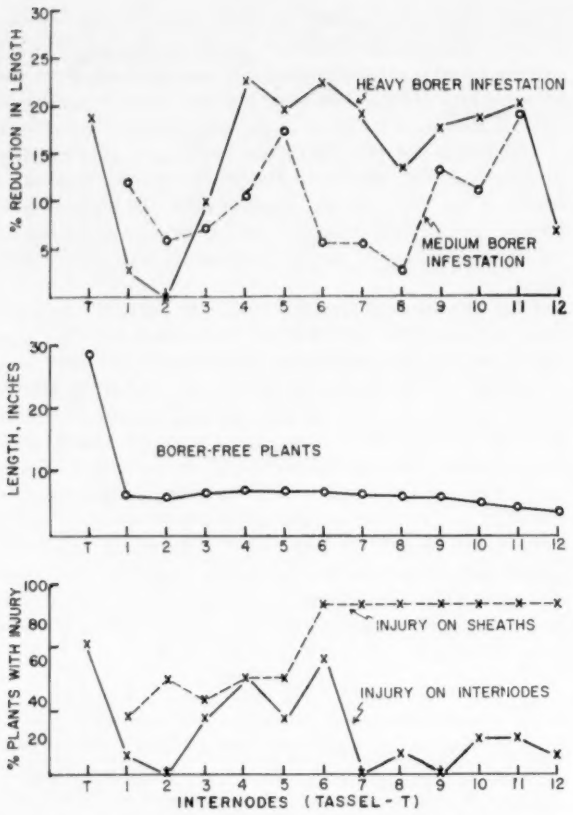

FIG. 4.—Upper, per cent reduction in length of internodes of plants with two levels of borer infestation. Middle, length of internodes of borer-free plants. Lower, borer injury on internodes and on leaf sheaths of plants with heavy borer infestation. Late planting WF9×M14, 1957 records.

that the distribution of the injury sites was irregular, but that it was by no means always correlated with the degree of reduction in the length of the internodes.

Further examination of the results of the two plantings, the two levels of borer infestation, and the 2 years, showed that, in general, the reduction in length was greater in the tassel and in the internodes near the tassel, and also in the internodes near the ear. Therefore, it seems logical to assume that the reduction was correlated with, among other things, the location of the sexual organs. This assumption may be supported by the fact that the development of the anthers on the tassel and of the kernels on the ear demands a large amount of nutrients, and that such demands usually overrule the needs for the growth of the vegetative parts. Thus any disturbance in the balance of nutrient supply owing to borer infestation would have a greater effect on the internodes near the sexual organs than on other internodes.

3. *The Time When Reduction in Length of Internodes Takes Place.*—In order to determine the time at which the reduction in plant height began to become apparent, the height of 30 borer-free plants and 30 plants with heavy borer infestation was checked at regular intervals during plant growth. Records were taken in the early planted susceptible WF9×M14 in 1955, 1956, and 1957. The per cent reduction is plotted, in figure 5, against (1) the tassel-bud ratio of the plants and (2) the average instars of the borers at the respective times. Tassel-bud ratio is a measure of plant development. It is the height of the tassel or "tassel bud" as expressed in per cent of the total plant height as proposed by Luckmann & Decker (1952). This ratio will be 100 when the tassel is completely extended and the plant has reached its maximum height.

The results show that the reduction in height caused by borer infestation increased gradually as the season advanced and as the plant grew. Regarding the time at which the reduction began to become apparent, the results of the three seasons varied. But, in general, it may be concluded that reduction in height began to show up at a stage of plant growth when the tassel-bud ratio was in the range of 40 to 60%, or at an average of 50%, *i.e.*, when the plants had reached half their maximum growth. Also, the reduction in height occurred in all years at the time when the borers were in the second instar or soon thereafter.

It is general knowledge that the first-brood borers begin to enter the stalk usually after they reach the third or later instar. The results discussed above suggest that the reduction in height was initiated before the borers entered the stalk and when only the leaf blades and leaf sheaths

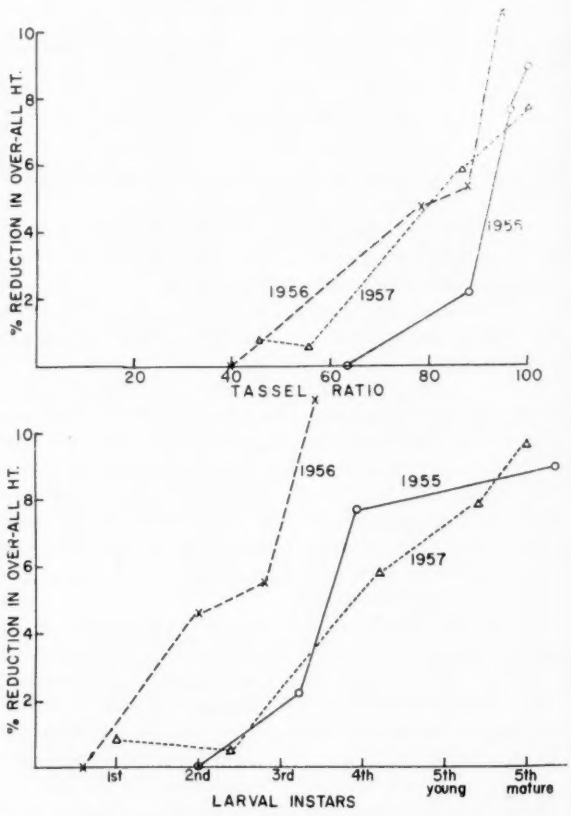

FIG. 5.—Upper, per cent reduction in the lengths of internodes of plants with heavy borer infestation as correlated with plant growth as expressed by tassel ratio. Lower, the same correlated with larval development.

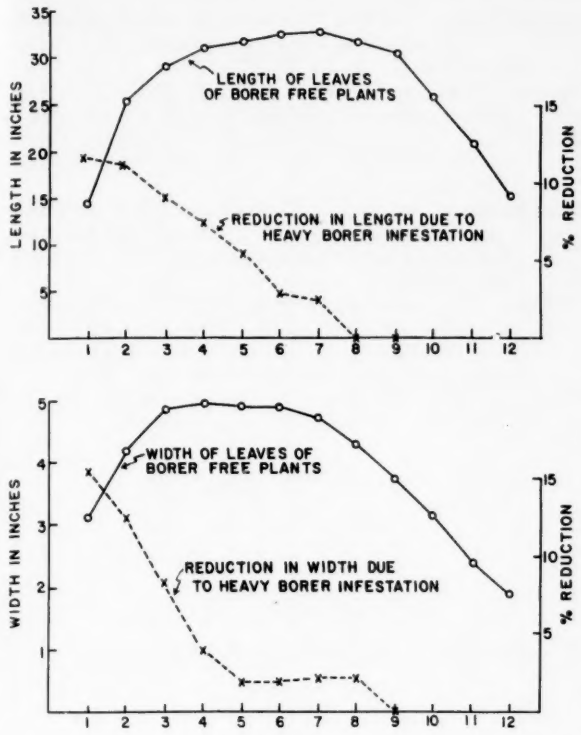
LENGTH IN INCHES
LENGTH OF LEAVES OF BORER FREE PLANTS
REDUCTION IN LENGTH DUE TO HEAVY BORER INFESTATION
% REDUCTION
WIDTH IN INCHES
WIDTH OF LEAVES OF BORER FREE PLANTS
REDUCTION IN WIDTH DUE TO HEAVY BORER INFESTATION
% REDUCTION

FIG. 6.—Length (upper graphs) and width (lower graphs) of leaves on borer-free plants, and the per cent reduction owing to borer infestation. Early planting WF9×M14, 1954 records.

had been injured. This phenomenon is especially interesting since the area of leaf tissues destroyed up to that time in these plots was no greater than 1% of the total leaf area.

4. *Reduction in Leaf Size.*—The effect of borer infestation on the growth of the leaves was investigated in the early planted susceptible WF9×M14 in 1954. The length and width of the individual leaves of plants with heavy borer infestation and those of borer-free plants were measured and compared. Averages of 10 plants of each type are given in figure 6. These results show that the length and width of leaves of borer-free plants were small at the base of the plant, increased toward the middle of the plant, and decreased again toward the top of the plant. This pattern of leaf size of normal plants confirms the findings of Anderson (1949). The results further show that the size of leaves above the eighth was adversely affected by borer infestation. The per cent reduction in both the length and the width was the greatest in the topmost leaf, and decreased progressively in the lower leaves. This pattern appears different from that of the internode growth, but can be explained on the same basis. It has been demonstrated (Sharman 1942) that the elongation of the leaf blade precedes the elongation of the subtending internode. At the time the elongation of leaf blade took place only the tassel would be in active growth and be demanding a great amount of nutrient. Thus the nearer the leaves are to the tassel, the more they are affected. On the other hand, when the elongation of the internodes took place at a later time, both the tassel and the ear would be in active growth, hence the internodes near both structures are affected.

5. *Delay in Tasselling and Silking.*—The effect of borer infestation on the dates of tasselling and silking was also studied in the four plantings (early and late WF9×M14, and early and late Oh43×Oh51A). During the period of tasselling and silking, the percentage of plants showing tassel and silk was checked at 2- to 3-day intervals. Such records were taken in 1956 and 1957 on borer-free plants as well as on plants with heavy borer infestation. The results as illustrated in figure 7 show that, in general, borer infestation delayed tasselling and silking in both single crosses.

GENERAL DISCUSSION.—The results presented show that borer infestation reduced the size of leaves and the length of internodes, and also delayed tasselling and silking of corn plants of borer susceptible single-cross WF9 ×M14 and borer resistant single-cross Oh43×Oh51A. Since these aspects of plant growth all involve the process of elongation, it may be concluded that borer infestaton reduces and delays the elongation of the tissues of the host plant.

The reduction in the length of internodes began before the borer entered the stalk and at a time when only the leaf blades were injured. Therefore the effect of borer infestation on the elongation of internodes must have been brought about by processes other than the physical destruction or obstruction of the vascular bundles in the stalk. This fact suggests that the borer feeding on leaf blades could have been responsible for or could have initiated some chemical change which reduces the normal process of growth, particularly elongation, of the inter-

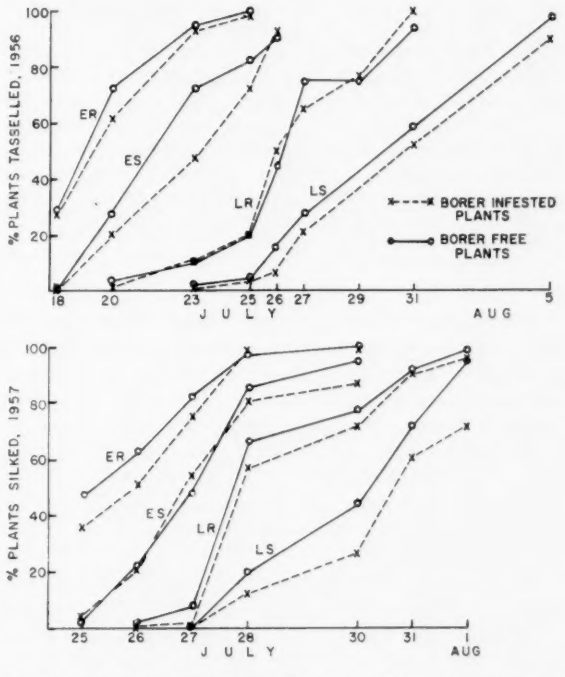

FIG. 7.—Tasselling (upper graphs) and silking (lower graphs) of plants free of borer and plants with heavy borer infestation. ER, early planting Oh43×Oh51A; ES, early planting WF9 ×M14; LR, late planting Oh43×Oh51A; LS, late planting WF9×M14.

nodes as well as of the leaf itself. The chemical changes may involve a phytotoxic secretion produced by the borers or by micro-organisms which may be associated with the borers.

It is logical to assume that the vegetative growth of corn plants affects ear growth. Therefore an understanding of the effect of borer infestation on vegetative growth may aid in the understanding of the effect of borer infestation on ear growth. The present study has uncovered enough facts to indicate the need for further studies along the lines of plant anatomy, plant physiology, and plant chemistry in association with studies on insect biology.

REFERENCES CITED

Anderson, Edgar. 1949. The corn plant of today. Des Moines.

Heimsch, B. C., and H. J. Stafford. 1952. Developmental relationships of the internodes of maize. Bull. Torrey Bot. Club. **79**: 52–58.

Everett, T. R., H. C. Chiang, and E. T. Hibbs. 1958. Some factors influencing populations of European corn borer, *Pyrausta nubilalis* (Hbn.) in North Central States. Minnesota Agric. Expt. Sta. Tech. Bull. No. **229**, 63 pp.

Luckmann, W. H., and G. C. Decker. 1952. A corn plant maturity index for use in European corn borer ecological and control investigations. Jour. Econ. Ent. **45**: 226–32.

Martin, J. N., and A. L. Hershey. 1935. The ontogeny of the maize plant—The early differentiation of stem and root structures and their morphological relationships. Iowa State Coll. Jour. Sci. **9**: 489–503.

Sharman, B. C. 1942. Developmental anatomy of the shoot of *Zea mays* L. Ann. Bot., n. s. **6**: 245–82.

Tracking Honey Bees in Cotton Fields with Fluorescent Pigments[1]

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ABSTRACT

A unique planting arrangement of rows of Pima S-1 and Pima 32 long staple cotton provided an opportunity for observing the transfer of fluorescent pigment by honey bees (*Apis mellifera* L.) between flowers of the two varieties. Although a preference was shown for Pima S-1, both varieties were visited and cross-visited. Pigments placed in Pima 32 flowers were about equally distributed to both varieties, but pigments placed in Pima S-1 flowers were distributed two to three times as frequently to Pima S-1 as to Pima 32 flowers.

There was no significant relationship between distance and number of marked blooms in the entire field, but this may have been due to the heterogeneous arrangement of the varieties. Tests using small areas within homogeneous plantings showed a highly significant distance effect.

During the summer of 1956 experiments were conducted at Sahuarita, Arizona, using fluorescent pigments for tracking honey bees (*Apis mellifera* L.) in long-staple cotton (*Gossypium barbadense* L.). The study was undertaken to obtain information on the activity of bees in a cotton field planted to Pima-32 and Pima S-1 varieties for hybrid cottonseed production. In contrast to Upland cotton, the long-staple plants produce larger, more brilliantly yellow flowers followed by late maturing bolls of three or four locks.

Attempts to track marked bees appear to have originated with the honey gatherers of antiquity. In 60 A.D. Columella, who compiled his information from Hyginus, Virgil, and Celsus, wrote of marking bees with "liquid oker" for the purpose of locating wild colonies. The problem was still apparent 30 years ago, when Park (1929) reviewed the literature and concluded: "Lack of a satisfactory method for marking bees has always been and still is one of the greatest obstacles to studying the life of the individual honey bee." In recent years suggestions for improved marking methods have included aerosol paints (Singh 1950) and aluminum dusts in hive-entrance dispensers (Nairn 1953). Stephens & Finkner (1953) and Thies (1953) treated cotton flowers with methylene blue dye to study its dispersal by pollinating insects.

MATERIALS AND METHODS.—In view of the success reported by Musgrave (1949, 1950) with fluorescein and Smith (1949–55, 1958) and Smith & Townsend (1951) with other fluorescent materials with ultraviolet light, fluorescent pigments were chosen for use in this investigation. The cotton flower is particularly suitable for studies with pigments, for it opens in the morning, closes in the evening, withers by the following morning, and the corolla soon drops off. With a little experience the day-old flowers can be distinguished from older ones on the basis of color, dryness, and ease of removal.

The 80-acre field used in this study (fig. 1) contained the following arrangements of Pima 32 and Pima S-1:

1-1—single rows of Pima 32 alternating with single rows of Pima S-1
2-2—pairs of rows of Pima 32 alternating with pairs of rows of Pima S-1
Mix—rows planted with a mixture of 1 lb. Pima 32 to 2 lb. Pima S-1 seed
Solid—rows planted with Pima S-1.

This unusual planting arrangement provided data on bee visitation not likely to be found in a homogeneous field and which may be of interest to cotton breeders and students of pollination.

Two methods of applying pigments were used. In three experiments 50 mg. of Helecon fluorescent pigment were

[1] Accepted for publication December 22, 1959.
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[3] In cooperation with the Arizona Agricultural Experiment Station.

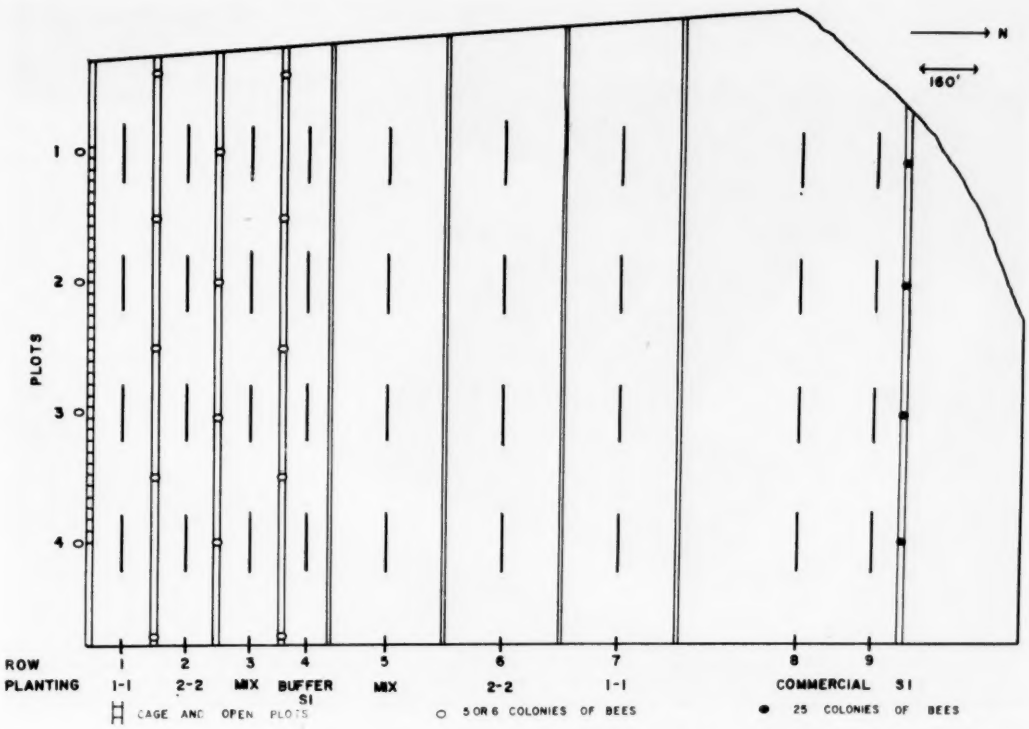


FIG. 1.—Layout of the 80-acre experimental field, showing method of planting Pima S-1 and Pima 32 long staple cotton, and plot and colony locations. Buffer and commercial were solid Pima S-1 plantings. Gene Carey ranch, Sahuarita, Ariz.

placed in a selected flower. Samples of flowers were then collected at various distances from the treated ones for examination. It was assumed that any pigment found in untreated flowers was carried there by the bees. In two other experiments the fluorescent pigment diluted with 9 parts of lycopodium spores was placed in a hive-entrance insert on a honey bee colony (fig. 2). With this device on the hive, the bees going to the field walked through the material and carried the powder adhering to their body hairs to the flowers they visited.

Samples of day-old flowers were collected the morning after treatment and examined under ultraviolet light in the laboratory. The detection of pigment in these flowers was evidence of visits by bees from the treated flowers or hive and thus served to trace their activity in the field. Refrigeration at 40° F. to avoid the need for immediate examination was not satisfactory, because of rapid decay and consequent difficulty in opening the flowers.

PIGMENTS APPLIED TO FLOWERS.—In the first experiment the pigment was applied to two flowers on a single Pima S-1 plant in the 2-2 planting. These two flowers were visited by 41 honey bees between 8 A.M. and 12:30 P.M. The next morning all flowers that had been open the previous day were collected from plants in the same row within 50 feet on both sides of the treated flowers and along a comparable distance in the adjacent 15 rows to the south. The results of the examination of these flowers are shown in figure 3. Clearly the bees had carried the pigment from the two treated Pima S-1 flowers to both varieties. However, the coverage was more thorough on the seven rows closest to the two treated flowers. Of 100 flowers collected, pigment was found in 80.

In a second experiment five Pima S-1 flowers in the 1-1 planting were treated with one pigment and five Pima 32 flowers in an adjacent row with another pigment. This procedure was repeated at four locations in the field. On a second trial at each location, the pigments were alternated. All flowers open on the day of treatment within a 30×40 foot area were collected. The treated flowers were located at the center of this area. Of the 2,451 flowers collected (1,204 Pima 32 and 1,247 Pima S-1), 1,282 showed either one or both pigments, as summarized below:

	Pima S-1	*Pima 32*
Pima S-1	677	316
Pima 32	38	50
Both	139	62
	854	428

The occurrence of Pima S-1 and Pima 32 flowers was in a ratio of 1.04:1, but twice as many Pima S-1 as Pima 32 contained pigment.

The flowers in each row of cotton were pooled for the analysis of variance[4] (table 1) and graphic presentation (fig. 4). Regardless of variety, pigment dispersion decreased significantly with distance, as substantiated by the nonsignificant D×O and R×D interactions in the analysis of variance. Pigments from the Pima S-1 flowers occurred with significantly greater frequency in all flowers examined, indicating a definite preference of honey bees for these flowers. The comparison of means

[4] The assistance of Henry Tucker, Statistician, College of Agriculture, University of Arizona, is gratefully acknowledged.

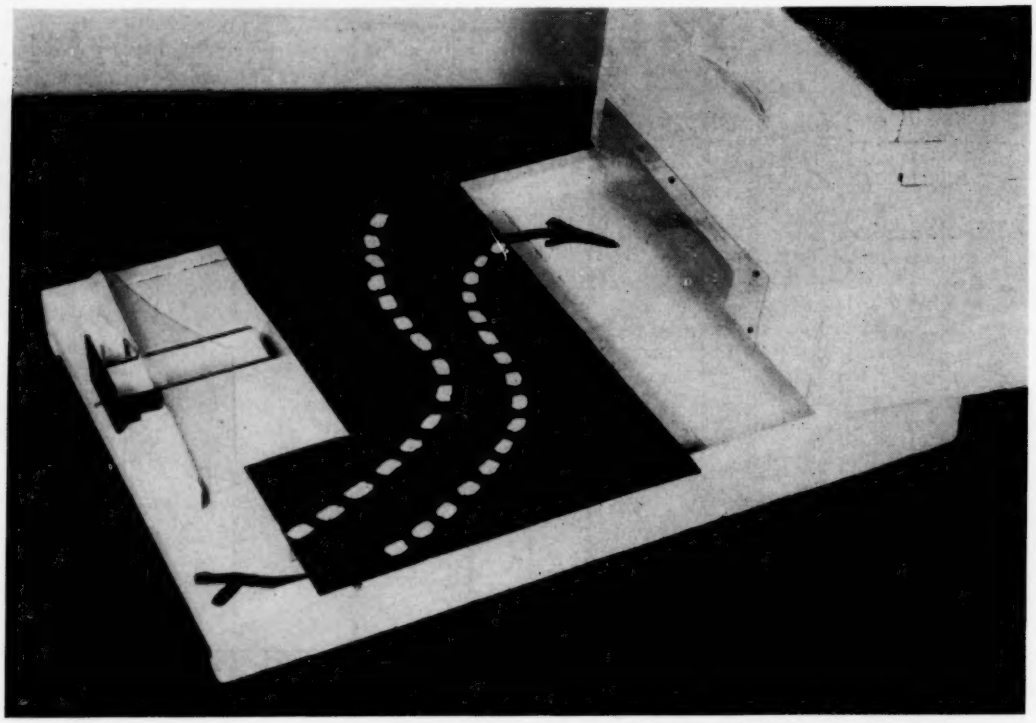


FIG. 2.—Hive-entrance insert with drawer partly pulled out. The hive body is set on the bottom board against the plastic shield to keep entering and exiting honey bees separated. A $1\frac{1}{4}\times7\frac{3}{4}$ inch opening, centered over the drawer, was cut from the lower edge of the hive body and covered with plastic.

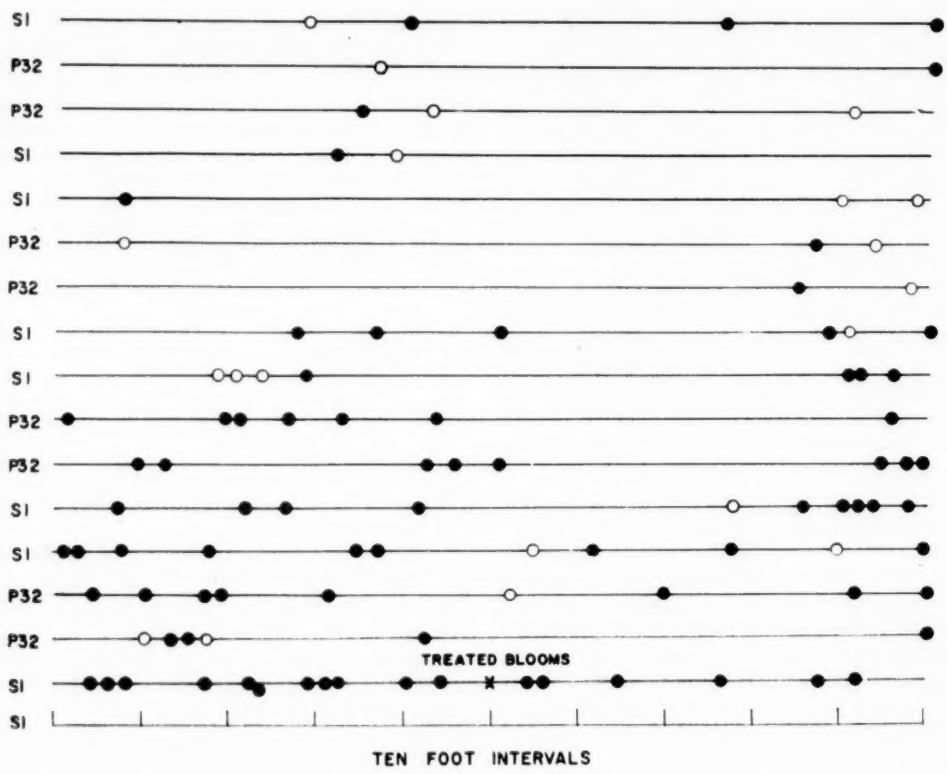


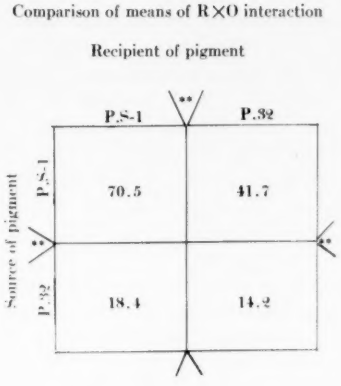
FIG. 3.—Cotton flowers (circles) collected from a 5,000-square-foot-area the day after treatment of two flowers (x) with fluorescent pigment. Solid circles represent flowers that received pigment from visiting honey bees.

Table 1.—Analysis of variance for data in Figure 4.

SOURCE OF VARIATION	d.f.	S.S.	M.S.	f.
Locations (4)	3	13,320.63	4,440.21**	26.84
Variety of flower receiving pigment (R)	1	5,591.17	5,591.17**	33.79
Distance (D)	4	3,491.34	872.84**	5.27
Variety of flower from which pigment was obtained (O)	1	32,032.02	32,032.02**	193.63
R×D	4	1,128.92	282.23	1.71
R×O	1	3,137.50	3,137.50**	18.99
D×O	4	436.13	109.03	.66
R×D×O	4	3,360.12	840.03**	5.08
Error	57	9,429.39	165.43	
Total	79	71,927.22		

** Highly significant.

Comparison of means of R×O interaction



(table 1) shows the relative pigment dispersion from the two sources as follows: (1) Pigments were moved 3.4 times more frequently from the Pima S-1 than from the Pima 32 flowers; (2) pigments from the Pima 32 flowers were distributed about equally (1.3:1) among both varieties; (3) pigments from the Pima S-1 flowers were distributed in the ratio of 1.7:1 in favor of this variety.

For the third experiment an area was chosen in the 1-1 planting near the center of the field. Stakes were set at 40-foot intervals along the center row, and in every 10th row ($33\frac{1}{3}$ feet) along radii extending 200 feet from the center in eight directions (fig. 5). Near the center stake three flowers of each variety were treated with pigments. These flowers were observed continuously from 9:45 A.M. to 3:45 P.M. and all honey bee visits recorded. The three Pima S-1 flowers received 131 visits while the three Pima 32 flowers received 68, a ratio of approximately 2:1.

On the following day samples of 10 flowers of each variety were collected near each of the 35 stakes and examined for fluorescent pigments. The percentage of marked flowers collected within the projected circles (fig. 5) represents the pigment distribution by 199 honey bees visiting the six marked flowers in the center. The greatest percentage of marked flowers occurred closest to the treated flowers, but some material was found as far as 200 feet away. Of the 350 flowers of each variety collected the following distribution of pigment was found:

	Pima S-1	*Pima 32*
Pima S-1	65	17
Pima 32	7	5
Both	12	4
	84	26

Pigment was distributed to Pima S-1 and Pima 32 in a ratio of 3.2:1, whereas the ratio of original honey bee visits to the marked flowers was 2:1. The secondary dispersal by other pollinators may have been a contributory factor but was not studied.

APPLICATION OF PIGMENT WITH A HIVE INSERT.—Two experiments were performed with a modification of the hive-entrance insert (fig. 2) developed by the Nova Scotia Agricultural College (Karmo and Vickery). The hive insert was placed on a single colony of bees near the southwest corner of the field. The day following treatment 25 withered flowers (open the day of treatment) were collected from each of 36 field plots and examined under ultraviolet light. "Plot" here refers to the 36 portions (150-foot long) of the nine rows indicated by number in figure 1. The results of these two experiments are shown in table 2.

The flowers collected in all the plots except the "mix" plantings were from Pima S-1 plants. Different numbers of plots and plots planted differently fell within the 500-foot intervals from the treated hive to the midpoints of the plots; consequently the data are not strictly comparable. For example, no solid Pima S-1 plot was represented within 0 to 500 feet and only the solid Pima S-1 plots within 2,000 to 2,500 feet.

Plot 1 in row 1 (108 feet from the treated hive) had 66% of the flowers containing pigment, the highest of any single plot. The next highest was plot 4 in row 4, with

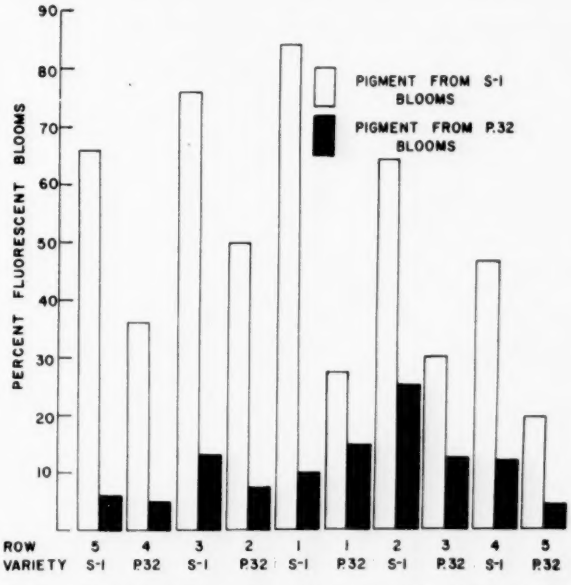


FIG. 4.—Per cent of cotton flowers in an area 30×40 feet visited by honey bees marked with two colors of fluorescent pigment applied to five flowers each of Pima S-1 and Pima 32 long staple cotton.

60.5% 1,266 feet from the hive. The two farthest plots from which samples were obtained were plot 3 in row 8 (2,079 feet) and plot 2 in row 9 (2,386 feet), with 44 and 17.5% of marked flowers, respectively.

Statistical analysis showed no significant relationship between distance and number of flowers containing pigment in contrast to analysis of data from the small areas studied (see table 1). Means for the solid Pima S-1 and 2-2 plantings (43.1 and 41.3, respectively) compared with those for "mix" and 1-1 plantings (25.0 and 26.3, respectively) apparently indicated a preference for the former arrangement (see table 2).

DISCUSSION.—It is not known to what degree honey bees are responsible for the transfer of pollen from one cotton flower to another. In our tests pigment was placed in the base of the flower as well as on the anthers. Kearney (1923) observed the readiness with which honey bees enter unopened flowers early in the morning, when they are more likely to rub against the stigma—"later, . . . the bees usually crawl down the inside of the petals without touching the stigmas or stamens and make their exit in the same manner." He noted that *Melissodes*, unlike the honey bee, commonly crawls over the stigmas and stamens of open flowers. The secondary dispersal of pigment from a flower after deposition by a honey bee will depend upon the number of all insects that visit such flowers. No attempt was made to measure the degree to which floral visitors other than honey bees were responsible for such dispersal of pigment.

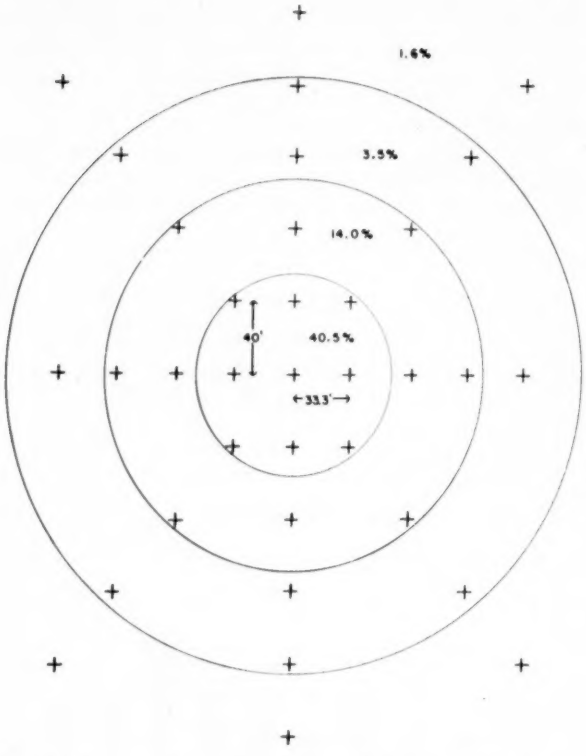


FIG. 5.—Layout of sampling stations (+) in 106,400-square-foot-area. Ten flowers from Pima S-1 and 10 flowers from Pima 32 plants closest to each sampling station were collected the day following treatment. The combined percentage containing pigment is given for the projected areas.

Table 2.—Percentage of flowers marked with fluorescent pigment by honey bees from one hive (in southwest corner of field) equipped with an entrance insert.[a]

Distance of Midpoint of Plot from Treated Hive (Feet)	Percentage of Flowers Containing Pigment				
	1-1	2-2	Mix	Solid P.S-1	Mean
0– 500	53.0 (2)[b]	43.0 (2)	40.0 (1)	—	45.3
500–1000	10.0 (1)	28.0 (1)	24.5 (4)	49.7 (3)	28.1
1000–1500	16.0 (1)	38.3 (4)	14.4 (3)	60.5 (1)	32.3
1500–2000	21.1 (4)	56.0 (1)	—	—	38.6
2000–2500	—	—	—	19.0 (8)	19.0
Mean	25.0	41.3	26.3	43.1	

[a] Two replications of 25 flowers were collected from each of the 36 field plots shown in figure 1.

[b] Figures in parentheses indicate the number of plots found within the distance interval.

Results obtained with this marking technique may not be directly applicable to pollen dispersal, as pointed out by Thies (1953): "It is not necessarily true that pollen from the first row would affect pollination in all rows, as is suggested by the dispersal of the dye. The dye particles are smaller and drier than pollen grains and differences in gathering and disseminating the two substances by bees could be expected. Nevertheless, the thoroughness of the bees in flower visitation is demonstrated by the results obtained."

Caution precludes drawing any conclusions concerning foraging distance from observations made with the hive-entrance insert without taking note of the alternate row and mix planting arrangements of the field in which they were made. The plots farthest from the marked hive were in solid Pima S-1 (rows 8 and 9), and the apparent greater attractiveness of this variety may have caused the honey bees to forage farther than they would in a uniformly planted field. The presence of 212 other colonies in the field used for these tests may have affected the results also. There is no adequate information concerning the effect of competition between bees upon the size of foraging area of either individual bees or colonies (Ribbands 1953).

Analysis of data obtained from tests made in small areas with homogeneous plantings showed high significance for distance of pigment dispersal from treated flowers. Analysis of data from tests made on the entire heterogeneous field showed no linear correlation between distance from the treated hive and the number of flowers containing pigment.

REFERENCES CITED

Columella, L. J. M. 60 A.D. Of husbandry in twelve books and his book concerning trees (Eng. trans. 1745). A. Millar, London.

Karmo, E. A., and V. R. Vickery. [No date.] The place of honey bees in orchard pollination. Nova Scotia Dept. Agric. and Marketing, Mimeo Circ. Serv. **67:** 12 pp.

Kearney, Thomas H. 1923. Self fertilization and cross fertilization in Pima cotton. U. S. Dept. Agric. Bull. **1134:** 66 pp.

Musgrave, A. J. 1949. The use of a fluorescent material for marking and detecting insects. Canadian Ent. **81**(7): 173.

Musgrave, A. J. 1950. A note on the dusting of crops with fluorescein to mark visiting bees. Canadian Ent. **82**(9): 195–6.

Nairn, R. G. 1953. A new method of marking bees. New Zealand Jour. Agric. **86**(6): 529.

Park, O. W. 1929. Time factors in relation to the acquisition of food by the honeybee. Iowa State Coll. Agric. Expt. Sta. Res. Bull. **108**: 226 pp.

Ribbands, C. R. 1953. The behaviour and social life of honeybees. 352 pp. Bee Research Assoc. London.

Singh, S. 1950. Behavior studies of honeybees in gathering nectar and pollen. New York (Cornell) Agric. Expt. Sta. Mem. **288**: 57 pp.

Smith, M. V. 1949–55. [Several titles in] Progress Reports. Legume Research Committee in Ontario. Ontario Coll. Agric., Agriculture Dept.

Smith, M. V. 1958. The use of fluorescent markers as an aid in studying the foraging behaviour of honeybees. Proc. 10th Internatl. Congr. Ent. **4**: 1063.

Smith, M. V., and Townsend, G. F. 1951. A technique for mass-marking honeybees. Canadian Ent. **83**(12): 346–8.

Stephens, S. G., and M. D. Finkner. 1953. Natural crossing in cotton. Econ. Bot. **7**(3): 257–69.

Thies, S. A. 1953. Agents concerned with natural crossing of cotton in Oklahoma. Agron. Jour. **45**(10): 481–4.

Natural Control of Florida Red Scale on Citrus in Florida by Predators and Parasites[1]

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ABSTRACT

This study was initiated after observations and grove studies had shown that Florida red scale (*Chrysomphalus aonidum* (L.)) was adequately controlled by natural factors. Although some of the predators and parasites have not been thoroughly evaluated, data collected so far indicate that parasitic hymenoptera represent the critical factor in natural control. The influence of weather and chemical control measures on natural control have been investigated, but the direct effect of weather on the scale and the scale's food supply has not been studied.

Two parasitic fungi are known to infect the scale but they are believed to be unimportant factors in natural control owing to their strong host density dependency and erratic incidence in scale infestations. Twenty-one species of predators are known to feed on Florida red scale, but only six are common, widely distributed, scale-feeding species. Evaluation studies have not been completed, but all attempts to associate individual predator populations with decreases in scale infestations have failed. Therefore, it seems likely that predation is not a critical natural control factor, although predators probably have a leveling effect on scale infestations. Eleven different parasitic wasps attack the scale in citrus groves. Four primary parasites and one hyperparasite are common to abundant. Three of these primaries attack male and second-stage female scales; one attacks mature female scales. The hyperparasite attacks *Prospaltella aurantii* How., one of the second-stage parasites. High rates of scale mortality result from parasitism, and evaluation studies have demonstrated a decided negative relationship between parasite populations and rates of parasitism, and intensity of scale infestations.

Studies on parasite incidence and abundance, effects of parasitism on scale infestations, and factors influencing parasite efficiency have indicated that *Pseudhomalopoda prima* (Gir.), the parasite attacking mature female scales, is the critical parasite species. Absence from or reduction of the species in Florida red scale infestations invariably results in outbreak infestations. The most important supporting parasite species appear to be *Aspidiotiphagus lounsburyi* (B. & P.) and *Prospaltella aurantii*, which attack male and second-stage female scales.

Florida red scale, *Chrysomphalus aonidum* (L.), is considered the second most important armored scale insect in Florida citrus groves. Although it is found on citrus throughout the State, it is more common in the Central and South Central citrus areas. According to figures obtained by the insect survey program (Pratt 1952), it is found in 14 to 75% of all groves. Injurious infestations may occur at any time of the year, but populations are generally larger in the fall and winter, with the winter infestations usually more severe and damaging. Infestations are restricted to the fruit and leaves, and the greatest density of scale frequently occurs on outside foliage and fruit. One or two limbs of a tree will often be more heavily infested than the rest of the tree.

Although Florida red scale occurs in abandoned and unsprayed groves, populations seldom reach high levels under such conditions. Infestations causing severe defoliation and fruit drop are nearly always confined to groves where some type of insecticide or acaricide treatment is applied. A study of the biologic factors influencing Florida red scale populations was initiated in 1955 in an effort to determine the causes of such infestations. As the biology of the scale is well known (Mathis 1941, 1947, Griffiths & Thompson 1948, 1949, 1949A), the present investigations were directed toward a determination and evaluation of predators and parasites.

BIOLOGIC FACTORS CONSIDERED.—Parasitic fungi, predatory arthropods, and parasitic hymenoptera have been observed and recorded attacking Florida red scale on citrus.

PARASITIC FUNGI.—One parasitic fungus, the chytrid *Myiophagus* sp., has been recorded attacking second- and third-stage Florida red scale (Fisher *et al.* 1949, Fisher

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1950A). A second parasitic fungus of the genus *Hirsutella* Pat. has been observed associated with dead crawlers during the course of the present study which condition apparently had not been recorded heretofore. A previously recorded abnormal condition of second- and third-stage scale, referred to as brown-spot disease (Muma 1955A), is now believed to be an indication of abnormal physiology following freezing or dehydration.

Chytridiosis, the disease condition caused by *Myiophagus* sp., does not cause mortalities consistently in Florida red scale. Although the disease and causal organism have been found in the scale throughout the citrus growing areas of the State, an epizootic develops only occasionally. As is usually true with a disease organism, a high host density is a necessary element for dissemination. In the case of chytridiosis the organism is waterborne (Fisher *et al.*, 1949), and rains, heavy dews, or fogs must occur concurrently with high host density before disease incidence of epizootic proportions is possible. Even when these controlling factors do occur simultaneously, chytridiosis still does not develop invariably, so it must be assumed that Florida red scale is not as adequate a host as purple scale, *Lepidosaphes beckii* (Newm.).

Hirsutella is not consistently associated with Florida red scale crawler abundance. Although species of *Hirsutella* described from purple scale crawlers and citrus rust mites, *Phyllocoptruta oleivora* (Ashm.) (Fisher 1950) have not been proved to be parasitic, they are believed to be indicators of specific diseases (Fisher 1950, 1950A). Mortalities associated with these described fungi increase with an increase in host numbers indicating a strong host density dependency. Such is not the case with the *Hirsutella* associated with Florida red scale; even when crawler numbers are high, *Hirsutella* incidence is low. Occasionally numerous crawlers exhibiting hyphae, phialides, and spores will be observed on sample leaves, but no consistent relationship has been noted. The fungus must therefore be considered of minor importance as a natural control agent.

Therefore, parasitic fungi, although recognized as occasionally effective factors, do not appear to play an important role in the natural control of Florida red scale.

PREDATORY ARTHROPODS.—Many predators have been reported to feed on Florida red scale. *Chilocorus stigma* (Say), *Exochomus marginipennis childreni* (Muls.), *Cycloneda sanguinea* (L.), *Hippodamia convergens* G.-M., *Epitragodes tomentosus* (Lec.), *Chrysopa* spp., and predatory mites were the first recorded (Watson 1918). *Olla abdominalis* var. *sobrina* Csy. (now known as var. *plagiata* Csy.) was later added to the list (Watson & Berger 1926). Recent additions include *Chrysopa lateralis* Guer. (now known as *cubana* Hagen) and *Hemisarcoptes malus* (Shimer) (Mathis 1947) and *Microweisea coccidivora* (Ashm.), *Aleurodothrips fasciapennis* Franklin, *Typhlodromus peregrinus* Muma, and *Cosmolaelaps* sp. (now known as *Zercoseius* sp.) (Muma 1955A). When these recorded predators were added to those observed and studied during this investigation, the list of predatory arthropods cited in table 1 was obtained. Of the 21 species listed in the table, 13 are common or abundant in Florida citrus groves and have warranted attention during this study.

Four lady beetles were investigated because they occur most commonly. One of these, *Cycloneda sanguinea*, was found to feed primarily on aphids; another, *Olla abdominalis* var. *plagiata*, rarely attacks armored scales, seemingly preferring mealybugs and soft scales. *Chilocorus stigma*, the twice-stabbed lady beetle, has previously been shown (Muma 1955C) to attack this scale, but does not seem capable (as an isolated factor) of reducing scale populations. The remaining species, *Microweisea coccidivora*, the little scale-eating lady beetle, has been reported (Muma 1955B) to feed on and apparently prefer the scale as a host. It has been demonstrated further (Muma 1956) that the species will complete a life cycle in the same length of time as the scale while utilizing the scale as a host. The survey conducted during this investigation indicated that this lady beetle is equally as common as the twice-stabbed lady beetle in Florida red scale infestations. Comparatively high mortalities were obtained, however, when the species was reared on the scale in the laboratory (Muma 1956), whereas low mortalities of the twice-stabbed lady beetle were obtained (Muma 1955C). This fact indicates that the little scale-eating lady beetle may feed on other hosts. Additional studies will have to be made before its relationship to the natural control of Florida red scale can be determined adequately.

Darkling beetles of several species are commonly associated with heavy scale infestations. In most cases they are found around or under large clumps of scale and trash where they appear to be feeding on dead and decaying materials. For this reason it is believed that the *Epitragodes tomentosus* recorded by Watson (1918) is probably a scavenger and of no importance as a natural control factor.

Only four of the seven green lacewings found to feed on Florida red scale were common enough to be considered. *Chrysopa bimaculata* McCl. completes a life cycle in 35 to 41 days on a diet of the scale, *Chrysopa cubana* Hagen in an average of 32 days, *Chrysopa interrupta* Schn. in 18 to 43 days, and *Chrysopa* sp. (undet.) in 29 to 32 days (Muma 1959). A special study of *C. cubana* has demonstrated that Florida red scale is probably a regular, if not a major, part of the diet of the species (Muma 1957). Despite these findings, however, no consistent correlation has been obtained between chrysopids and scale populations. There is no question that chrysopids, particularly *C. cubana* and *C. interrupta*, both of which are abundant in scale infestations, play a role in the natural control of this scale. Until controlled studies eliminating other factors have been made, however, the importance of that role cannot be fully determined.

Aleurodothrips fasciapennis, the brown and yellow thrips, is commonly associated with this scale (Muma 1955A). It reaches its peak of population in the late winter and spring. Thrips have been sustained on a diet of red scale in the laboratory, but all attempts to obtain a complete life cycle on the restricted diet have failed. Further, analyses of populations under grove conditions have resulted in correlations with purple-scale and whitefly infestations. Under these circumstances it appears that this predatory thrips is probably not a significant factor in the natural control of Florida red scale.

Three of the five species of predatory mites known to attack this scale are sufficiently common to have war-

Table 1.—Predatory arthropods known to feed on Florida red scale, *Chrysomphalus aonidum* (L.) on citrus in Florida.

PREDATOR	STAGE HOST ATTACKED	REMARKS AND NOTES
COCCINELLIDAE		
Chilocorus stigma	All	Primarily a scale feeder, abundant.
Cycloneda sanguinea	?	From *litt.*—primarily an aphid feeder, common.
Exochomus marginipennis childreni	All	Omnivorous feeder, uncommon.
Hippodamia convergens	?	From *litt.*—primarily an aphid feeder, rare.
Lindorus lophantae	All	New record for state—a scale feeder, rare.
Microweisea coccidivora (Ashm.)	All	Primarily a scale feeder, abundant.
Olla abdominalis var. *plagiata*	?	Primarily a soft scale feeder, common.
TENEBRIONIDAE		
Epitragodes tomentosus	?	From *litt.*—probably a scavenger, common.
CHRYSOPIDAE		
Chrysopa bimaculata	Young and mature scales	Feeds on scale in grove and laboratory, common.
C. cubana	Young and mature scales	Feeds on scale in grove and laboratory, abundant.
C. interrupta	Young and mature scales	Feeds on scale in grove and laboratory, abundant.
C. plorabunda Fitch	Young and mature scales	Reared on scale in laboratory, rare.
C. rufilabris Burm.	Young and mature scales	Reared on scale in laboratory, rare.
Chrysopa sp. (undeter.)	Young and mature scales	Reared on scale in laboratory, common.
Nodita floridana Banks	Young and mature scales	Fed on scale in laboratory, uncommon.
PHLOEOTHRIPIDAE		
Aleurodothrips fasciapennis	?	Consistently associated with scale, abundant.
HEMISARCOPTIDAE		
Hemisarcoptes malus	Eggs and crawlers	Well known scale predator, uncommon.
PHYTOSEIIDAE		
Amblyseius quadripilis	Eggs and crawlers	Fed on scale in laboratory, common.
Typhlodromus peregrinus	Eggs and crawlers	Fed on scale in laboratory, abundant.
ACEOSEJIDAE		
Zercoseius sp.	Eggs and crawlers	Fed on scale in laboratory, uncommon.
RAPHIGNATHIDAE		
Mediolata sp.	Crawlers	Fed on scale in laboratory, common.

ranted study. *Amblyseius quadripilis* (Banks), *Typhlodromus peregrinus* (Muma 1955), and *Mediolata* sp. have all been fed experimentally with eggs and crawlers in the laboratory but have failed to produce eggs and young on this food. Under grove conditions all three predators have been correlated with various infestations of mites, which they are now believed to utilize as food. Their observed capability of feeding on scale eggs and crawlers indicates, however, that these mites could utilize this scale as a host in the absence of more suitable food.

Therefore, predatory arthropods attacking Florida red scale fall into three classes insofar as natural control is concerned. The first class includes those species that are rare and uncommon in number of individuals or distribution; eight of the presently recorded predators are in this category. The second class includes those species that feed primarily on other hosts or food; nine predators are in this category. The third class includes common or abundant scale-feeding species that seem incapable of reducing infestations of the scale; six predators are in this category. It is probable that the overall effect of predation, whether rare, incidental, or inadequate as a single factor, is a general reduction in host population. The determination of such reduction must, however, await the discovery and use of better methods of investigation and analysis.

PARASITIC HYMENOPTERA.—Prior to the present study, four species of parasitic hymenoptera had been recorded attacking Florida red scale in Florida. *Aspidiotiphagus lounsburyi* (B. & P.) and *Pseudhomalopoda prima* (Gir.) were recorded in connection with a biological study of the scale (Mathis 1947). A third species, *Prospaltella aurantii* How., was recorded during investigations of scale outbreaks following applications of DDT (Griffiths & Thompson 1947). The fourth species, *Aspidiotiphagus citrinus* (Craw.), was reported as a factor in the natural control of the scale (Muma 1955A). In the present study the first three species have been confirmed as common parasites of the scale. The fourth species has not been recovered, however, and it is possible that the record is spurious. *A. citrinus* is a common parasite of male and second-stage female purple scale and probably early Florida red scale rearings were contaminated with purple scale.

A statewide survey of hymenopterous parasites attacking Florida red scale was conducted during the present investigation. Infested leaves were collected from all areas and returned to the laboratory where parasites were reared directly from the infested leaves and from isolated stages and individuals of the scale. Data obtained from these rearings were utilized in developing figure 1 and tables 2 and 3.

Altogether, 11 different species of parasitic wasps have been found associated with infestations of Florida red scale. Of these, five have been primary or direct parasites and three secondary or indirect parasites. Three species

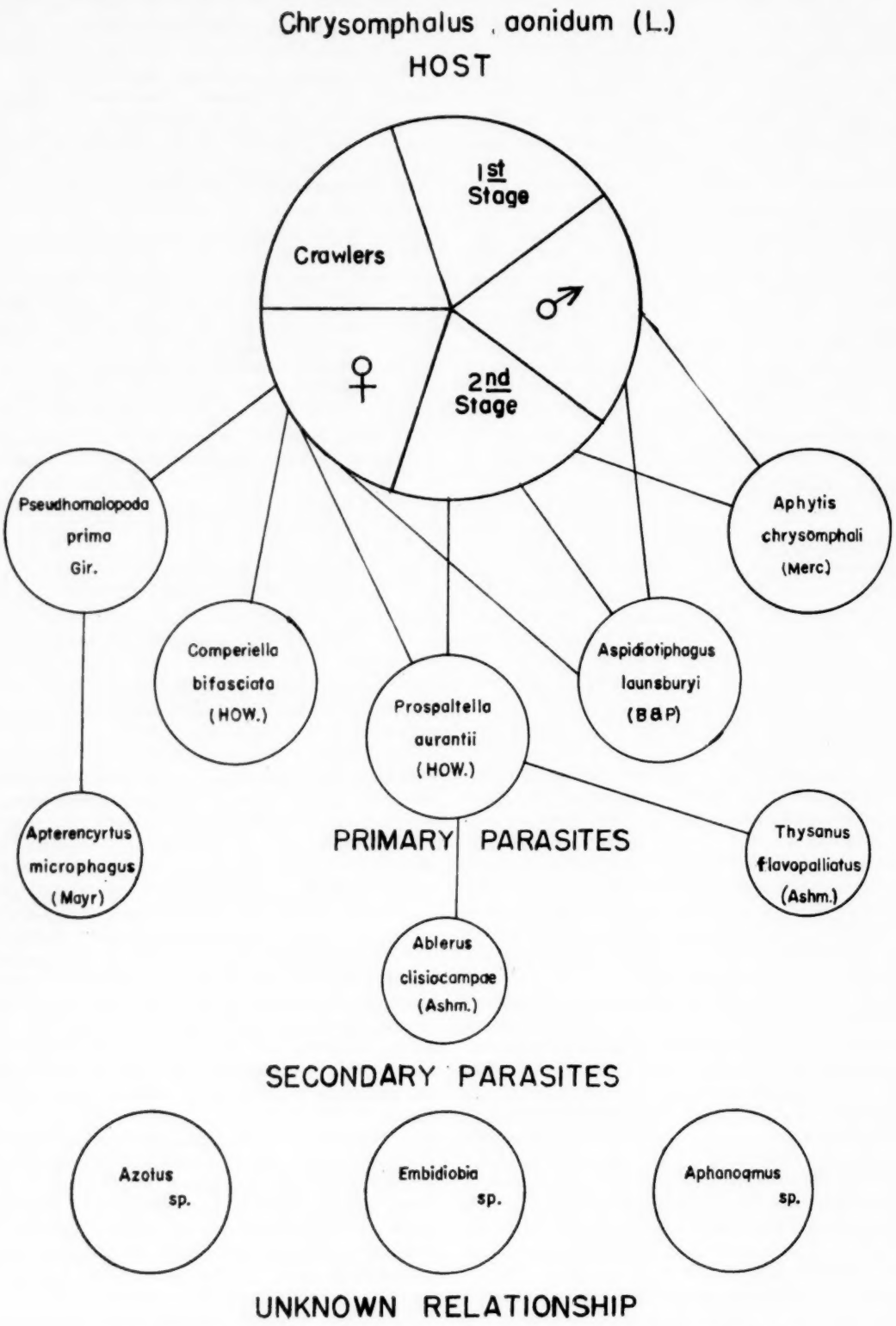

Fig. 1.—Diagram of the relationship of known parasitic wasps that attack Florida red scale.

Table 2.—Percentage of parasite population represented by each of the common hymenopterous parasites of Florida red scale in each season and in each citrus growing area.

SEASON OR AREA	PERCENTAGE OF PARASITE POPULATION FOR COMMON SPECIES					NO. SAMPLES
	Prospaltella	*Pseudhomalopoda*	*Aspidiotiphagus*	*Aphytis*	*Thysanus*	
North	49.89	13.63	21.11	10.59	4.78	36
Central	40.93	10.71	37.61	1.45	9.29	47
Southern	78.20	4.39	14.64	0.24	2.53	41
East coast	1.44	12.71	83.36	0.78	1.72	29
West coast	6.89	11.49	73.95	6.71	0.95	15
Totals	13,781	2,930	12,548	764	1,381	
Per Cent Total Population	43.88	9.33	39.96	2.43	4.40	
Winter	64.94	4.85	23.37	1.58	5.25	41
Spring	45.84	18.61	26.65	2.64	6.25	26
Summer	57.35	12.40	24.48	2.24	3.53	38
Fall	27.55	7.64	58.10	2.86	3.84	63
Totals	13,781	2,930	12,548	764	1,381	
Per Cent Total Population	43.88	9.33	39.96	2.43	4.40	

have not been taken in stage or individual scale rearings and their relationship to the ecology of the scale is unknown. Figure 1 shows the complex relationship of these parasites to the various stages of the scale. As can be seen, only crawlers and first-stage scale are not attacked by parasitic wasps. Females are subject to parasitism by four species, immatures by three, and males by two. Three secondary or hyperparasites attack the primaries, but only two of the primaries are subject to hyperparasitism. Among the primaries only one, *Comperiella bifasciata* How., is uncommon and not worthy of consideration. On the other hand only one of the secondaries, *Thysanus flavopalliatus* (Ashm.), is sufficiently common to be of importance. Table 2 shows the percentage of the parasite population represented by each of the five common species. The other six species represented less than 1% of the total parasite population.

Prospaltella aurantii, the most abundant species, primarily parasitizes immature or second-stage female scales. Occasionally second-stage males and adult females will be attacked, but the rate of successful parasitism is negligible. Second-stage female scales are highly parasitized by the species, with the observed rate of parasitism normally varying from about 2% to more than 90% with an average of 27.1%. The highest rate of parasitism observed for the species was 97.5%. This tiny brown and yellow eulophid is an internal parasite; the egg is laid inside of the scale body, the larva develops internally, pupation takes place within the hardened shell-like mummy of the host, and emergence is through an oval exit hole usually located laterally to the axis of the host body.

Aspidiotiphagus lounsburyi, the second most abundant species, parasitizes immature male and female scales, adult females, and subadult males. Subadult males are more highly parasitized by the species with the observed rate of parasitism varying from 1.5 to 55.5% with an average of 19.6%. Immature scales are the next most highly parasitized with the observed rate of parasitism varying from 3.7 to 37.0% around an average of 14.7%. Adult females are attacked the least with observed rate of parasitism usually negligible, but occasionally increasing to about 15%. This tiny yellow and brown species, also an eulophid, is an internal parasite; the egg is laid inside the scale body, the larva develops internally, pupation takes place within the cellophanelike mummy of the host, and emergence is through an oval exit hole usually located near the cephalic region of the host body.

Pseudhomalopoda prima, the only common species attacking mature female scales, apparently can complete development only in large nearly mature or mature females. The observed rate of parasitism normally varies from 2.3 to 24.8% around an average of 8.2%. The highest rate of parasitism observed for the species was 82.4%. This moderate-sized metallic-green encyrtid is also an internal parasite; the egg is laid inside the scale body, the larva develops internally, pupation takes place within the parchmentlike mummy of the host, and emergence is through a relatively large, irregularly oval exit hole that has no apparent polarity with the host body.

Aphytis chrysomphali (Mercet) is the least common of the primary parasites attacking this scale. Subadult males and second-stage females are the only stages parasitized by this species. No observed rates of parasitism have been obtained for the species, but it occurred in 36.3% of the infestations surveyed and in isolated groves represented as much as 38% of the parasite population. This tiny golden eulophid is the only external parasite

Table 3.—Seasonal and geographic incidence of common hymenopterous parasites in infestations of Florida red scale in Florida citrus groves, 1955–1958.

AREA OR SEASON	PERCENTAGE FLORIDA RED SCALE INFESTATIONS WITH					NO. OF SAMPLES
	P. aurantii	*A. lounsburyi*	*P. prima*	*A. chrysomphali*	*T. flavopalliatus*	
East coast	55.2	86.2	96.6	41.4	20.7	29
North central	83.3	69.4	83.3	58.3	44.4	36
Central	95.7	91.5	95.7	34.0	76.6	47
South central	97.6	68.3	85.4	14.6	36.6	41
West coast	80.0	86.7	80.0	40.0	26.7	15
Winter	82.9	80.5	85.4	31.7	43.9	41
Spring	88.5	84.6	92.3	34.6	53.8	26
Summer	76.3	63.2	81.6	28.9	36.8	38
Fall	90.5	87.3	95.2	44.4	49.2	63
All areas and seasons	85.1	79.8	89.3	36.3	45.8	168

known to attack Florida red scale in Florida. The egg is laid on the venter or side of the scale body, the larva develops externally, pupation takes place beneath the scale armor, and emergence is through an exit hole in the armor or out from under the loose armor.

Thysanus flavopalliatus, the only common secondary parasite, is hyperparasitic on *Prospaltella aurantii*. Its hyperparasitic habit has been deduced from the fact that it consistently rears from scale parasitized by *Prospaltella*. No specimens have been reared from scale parasitized by other primaries or from unique mummies. The species has been reared from 54.5% of *Prospaltella* populations. The highest rate of hyperparasitism obtained was 86.0%. A mean rate of hyperparasitism of 17.5% was obtained for the species in *Prospaltella*. No biological notes have been obtained for this tiny black and yellow thysanid.

Parasitic hymenoptera seem, therefore, to be the major biological factor influencing Florida red scale populations on citrus in Florida. This could be deduced from the fact that four common primary parasites are known to attack the scale causing high rates of mortality to several developmental stages. Such a deduction has not, however, been necessary as evaluation studies of several types have demonstrated a relationship between parasite populations and intensity of scale infestations.

PARASITE EVALUATION.—The importance of parasitic wasps to the natural control of Florida red scale on citrus has been determined by a survey of parasite incidence and abundance in scale infestations, a study of the effects of parasitism on scale infestations and an investigation of the effects of factors influencing the rate of parasitism.

Parasite Incidence and Abundance.—Prior to the present investigation, little was known concerning the geographic and seasonal incidence and abundance of the parasites attacking Florida red scale. General statements concerning high rates of parasitism during the winter months are all that have been recorded (Mathis 1947, Griffiths & Stearns 1947).

Incidence of the various species of parasites was determined on a statewide sample basis; that is, a parasite was determined as present in or absent from a scale infestation. The percentage of samples containing each species of parasite was utilized as the incidence figure in table 3.

On the basis of number of samples collected, it can be deduced that Florida red scale infestations are more common in the central and south-central citrus areas and during the fall and winter. Infestations are less common on the west coast and during the spring. In the 168 samples collected *Pseudhomalopoda prima* had the highest, most consistent incidence and *Aphytis chrysomphali* had the lowest, least consistent incidence of the primaries. The hyperparasite *Thysanus flavopalliatus* had the lowest incidence during the summer and in the warmer citrus areas, an incidence not entirely coincident with that of its host, *Prospaltella aurantii*.

Table 2 shows that although all species of parasites are present in all areas and during all seasons, there is considerable variation in relative abundance. *Prospaltella aurantii*, the most common parasite, comprises a much larger percentage of the parasite population in the central citrus areas than in the coastal areas and is most numerous in the winter and summer. *Aspidiotiphagus lounsburyi*, on the other hand, comprises the larger percentage of the population on the east and west coast, and during the fall. *Pseudhomalopoda prima* is relatively less abundant in the southern citrus area and during the fall and winter. *Aphytis chrysomphali* is relatively more abundant in the northern and west coast areas, but comprises about the same percentage of the parasite population the year around. The hyperparasite *Thysanus flavopalliatus* comprises a minor part of the total parasite population in all areas except the north and central with heavier populations occurring in the winter and spring.

Interpretation of these data on incidence and abundance indicate that literature citing high rates of parasitism in the winter probably refer mainly to *Prospaltella aurantii*. However, it should be noted that the heaviest, most consistent scale infestations occur in the central and south-central citrus areas which have proportionately more of this parasite than other areas. On the other hand, the data show a negative relationship between relative abundance of *Aspidiotiphagus lounsburyi* and scale infestations, indicating a more effective or more efficient parasite. It is more effective if the less frequent, less severe scale infestations are the result of greater parasite abundance, or more efficient if it is capable of maintaining relatively large populations where less severe, less frequent scale infestations occur. The reason for the low incidence and abundance of *Prospaltella* in the coastal areas has not been determined, but its abundance elsewhere seems to be influenced to some extent by the hyperparasite *Thysanus flavopalliatus*. It has also been noted that there is an inverse relationship between populations of *Prospaltella* and *Aspidiotiphagus*, indicating the possibility that the two species may be competitive.

Pseudhomalopoda prima, although not as abundant as *Prospaltella* or *Aspidiotiphagus*, exhibits a negative relationship to scale infestations in the various areas and seasons and is consistently present. These data indicate that the species is efficient and effective as a parasite. *Aphytis chrysomphali* populations have no distinct relationship to scale infestations and have a low incidence, indicating that the species probably is inefficient or ineffective.

Effects of Parasitism on Scale Infestations.—Muma (1955A) published the only previous data showing the direct effect of parasitism on Florida red scale infestations. At that time the statement was made that " . . . the only natural control factors oscillating with scale density are insect parasites."

Data showing the effect of parasitism on Florida red scale infestations have since been collected from several groves. These data have been obtained from samples consisting of 20 leaves selected at random in light to moderate infestations and 20 heavily infested leaves in severe infestations. Light infestations were sampled randomly in an attempt to demonstrate the effects of parasitism of second- and third-stage parasites on the population of third-stage female scales, representing the potential next generation. Samples of severe infestations were biased to heavily infested leaves so that a maximum number of individuals would be available for study of the effects of parasitism on both second- and third-stage scales.

Figures 2 and 3 show that, in light to moderate infestations of Florida red scale, an increase of healthy third-stage female scales is associated with or immediately followed by an increase in parasitized second- and third-stage scales. Increased parasitism is invariably associated with or immeditately followed by a decrease of healthy third-stage female scales. This relationship of parasitism to scale is consistent year after year in light to moderate infestations. It should be noted, however, that in samples of the size utilized in this study, parasitism could not be demonstrated in very light infestations.

In severe infestations the rate of parasitism is much higher than that shown in figures 2 and 3. However, severe infestations are rare in unsprayed citrus groves and such infestations in sprayed groves are usually treated promptly to avoid injury. For this reason there is a paucity of seasonal data on parasitism in severe infestations. Figures 4 and 5 present data obtained over a 3-month period in a severely infested pink grapefruit grove near Moss Bluff, Florida. As can be seen from these figures, the relationship of parasitized to nonparasitized scale is the same as that obtained in light to moderate infestations. These figures also show that second-stage parasites fluctuate with and apparently reduce populations of second-stage scales, while third-stage parasites are fluctuating with and reducing populations of third stage scale.

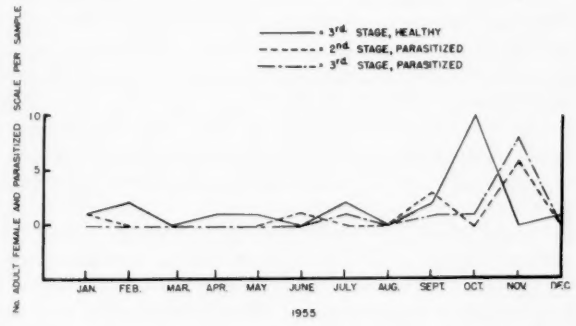

FIG. 2.—Healthy and parasitized female Florida red scale on 20-leaf samples from Valencia orange trees at Twin Lake, Florida, during 1955.

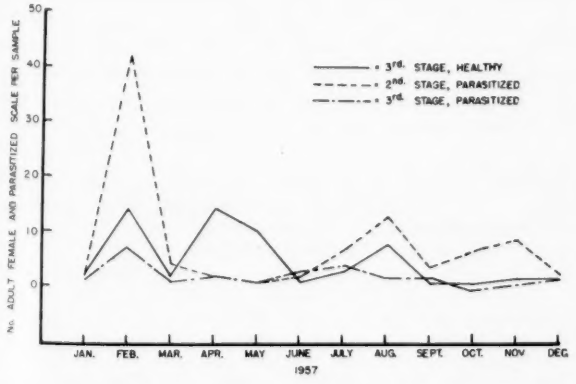

FIG. 3.—Healthy and parasitized female Florida red scale on 20-leaf samples from Marsh seedless grapefruit at Lake Alfred, Florida, during 1957.

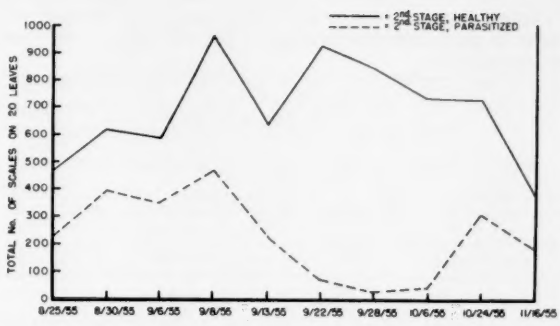

FIG. 4.—Healthy and parasitized second stage female Florida red scale on 20-leaf samples from August to November 1955, in a pink grapefruit grove at Moss Bluff, Florida.

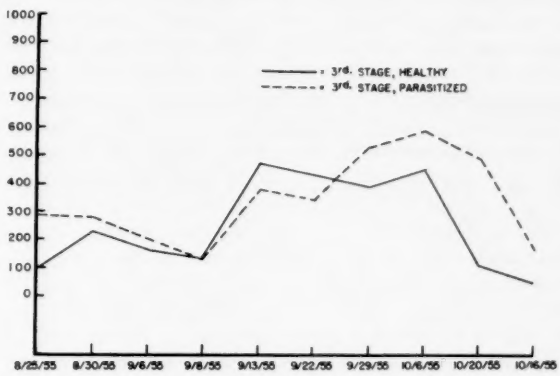

FIG. 5.—Healthy and parasitized third-stage female Florida red scale on 20-leaf samples from August to November 1955, in a pink grapefruit grove at Moss Bluff, Florida.

From the data obtained to date on the effects of parasitism on Florida red scale infestations, it seems that parasites are capable of effectively controlling this scale in unsprayed groves. Further, it appears that parasites can recover from the effects of insecticidal treatments in sprayed groves and eventually reduce and control severe infestations.

Factors Influencing the Rate of Parasitism.—During the course of the present study several factors have been found to influence rates of parasitism. These are sunlight, rain, low temperatures, and insecticides. Only one of these factors has been reported previously to influence parasitism and result in outbreaks of Florida red scale. Griffiths & Thompson (1947) and Griffiths & Stearns (1947) found that applications of DDT and oil and DDT reduced parasitism by *Pseudhomalopoda prima* and *Prospaltella aurantii*, resulting in increased infestations of Florida red scale.

The effects of sunlight on adult parasite populations were first observed in the fall of 1955. At that time adults of *Pseudhomalopoda prima* were being collected for shipment to Mexico and it was noted that more parasites were found on the lower surfaces of leaves and inside foliage than on the upper surfaces of leaves and outside foliage. Observations indicated that the parasites seemed to be

Table 4.—Relationship of Florida red scale location on leaves to parasitism on September 28, 1955, based on four 10-leaf samples.

Condition and Stage	Mean No. Scales per Leaf Surface		Per Cent per Stage		Per Cent per Surface	
	Upper	Lower	Upper	Lower	Upper	Lower
Nonparasitized second stage	22.3	18.8	2.0	1.7	3.8	3.6
Nonparasitized third stage	211.0	210.5	20.4	20.3	50.2	34.2
Parasitized second stage	569.0	510.0	50.8	45.5	96.2	96.4
Parasitized third stage	209.5	404.5	20.2	39.1	49.8	65.8

avoiding direct sunlight. Parasites on leaves inverted in the sun soon moved to the shaded side regardless of which surface was shaded. On the other hand parasites on leaves inverted in the shade showed no preference as to surface. In order to check the effect of this reaction on parasitism, four samples consisting of 10 randomly selected leaves were examined for rate of parasitism on upper and lower leaf surfaces. Table 4 presents the data resulting from these samples. *Pseudhomalopoda prima* parasitized third-stage scale on the lower leaf surfaces at a rate nearly twice that on the upper leaf surfaces, despite the fact that only 20% more third-stage scale occurred on the lower surfaces. The rate of parasitism of second-stage scale by the three common second-stage parasites was also determined, although no adult reaction to sunlight had been observed. In this case a slightly higher rate of parasitism was found on the upper leaf surfaces where the larger number of second-stage scales occurred. It should be noted that the infestation represented by the data in this table was quite severe. The effect of sunlight on parasitism is not so marked in light to moderate infestations. Possibly the tendency for Florida red scale infestations to be more severe on the periphery of the tree is due to this influence of sunlight on the rate of parasitism by *Pseudhomalopoda prima*.

Rainfall has also influenced the activity of adult parasites. During rains adult parasites move to the lower surfaces of inside leaves. Some of the differential parasitism of *Pseudhomalopoda prima* shown in table 4 may be due to rain. This is not believed likely, however, as rainfall occurs at irregular intervals and is of short duration.

Low temperatures have a striking effect on parasitism. Observations on the effects of low temperatures were first made in the fall of 1955. At that time collections of adult *Pseudhomalopoda prima* were placed in a refrigerator at 40° F. to hold for shipment; 12 hours at this temperature produced nearly 100% mortality. In December 1955, following cold defoliating winds, adults of this species were strikingly reduced in the grove under observation. Table 5 gives data collected from four heavily infested 10-leaf samples taken from this grove in January, 1956. The rate of parasitism by *Pseudhomalopoda prima* is significantly lower on outside foliage that was exposed to the cold winds, despite the presence of more third-stage scales. It should also be noted that more third-stage scale was frozen on outside foliage than on inside foliage. Table 6 shows the data collected from four consecutive randomly selected 20-leaf samples from the same grove in January, February, and March. These data show a reduced number of parasitized third-stage scale and a high percentage of frozen third-stage scale immediately following the cold winds. Forty days later the number of scales showing the effects of the cold winds was greatly reduced, but the rate of parasitism was approximately the same despite the fact that the number of healthy third-stage scales had increased greatly. The rate of parasitism did not significantly increase until the third sample, nearly 60 days after the first sample was taken. At the time of the fourth sample, however, the number of healthy third-stage scale was apparently reduced by the increased rate of parasitism.

Freezing temperatures during the winter of 1957–58 offered another opportunity for studying the effects of low temperatures on parasites. Table 7 shows comparative numbers of parasites reared from 28 infested leaf samples, consisting of 20 leaves per sample, collected prior to the December freezes and 28 samples collected during late December and January. Parasite numbers were strikingly lower in the postfreeze samples. Although much of this mortality can be attributed to direct freezing of the host, postfreeze counts on Florida red scale in 28 groves gave only a 55% mortality of second-stage scale and a 76% mortality of third-stage scale. The per cent reduction figures given in table 7 indicate that 15 to 40% of the parasite mortality can probably be attributed directly to the low temperatures.

Since the publication of Griffiths and Thompson's (1947) DDT study, observations in sprayed and unsprayed groves have led to the conclusion that chemicals other than DDT may have an effect on the rate of parasitism and subsequent level of Florida red scale infestations. Table 8 shows differences in rates of parasitism of third-stage Florida red scale by *Pseudhomalopoda prima* in adjacent sprayed and unsprayed test plots. This tend-

Table 5. Living, frozen, and parasitized third-stage Florida red scale on inside and outside of partially defoliated pink grapefruit trees at Moss Bluff, Florida, following cold winds in December 1955.

Location of Sample	Third-Stage Florida Red Scale					
				Per Cent		
	Living	Frozen	Parasitized	Scale Living	Scale Frozen	Scale Parasitized
			January 17, 1956			
Outside	234	249	17	46.8	49.8	3.4
Inside	181	77	113	48.8	20.8	30.5
Total	415	326	130	47.6	37.4	14.9
			January 23, 1956			
Outside	293	195	18	57.9	38.5	3.6
Inside	169	61	64	57.5	20.7	21.8
Total	462	256	82	57.8	32.0	10.3

Table 6.—Rates of parasitism of third-stage Florida red scale as related to frozen scale at Moss Bluff, Florida, following cold winds of December 1955.

Date (1956)	Living Third Stage	Frozen	Parasitized	Per Cent	
				Frozen	Parasitized
Jan. 5	36	468	38	86.3	7.0
Feb. 15	371	11	32	2.7	7.7
Mar. 2	208	5	39	2.0	15.5
Mar. 21	85	2	23	1.8	20.9

Table 7.—Number of parasites that emerged from Florida red scale samples before and after the December 1957 freeze in Florida.

AREA	NO. GROVES SAMPLED	TOTAL PARASITES				
		Prospaltella	*Pseudhomalopoda*	*Aspidiotiphagus*	*Aphytis*	*Thysanus*
		Before the freeze				
North	5	46	119	256	63	3
Central	4	399	80	175	0	10
South	6	171	88	603	12	11
East	6	2	164	1677	4	27
West	7	61	299	2207	220	23
Total	28	679	750	4918	299	74
		After the freeze				
North	5	17	0	38	6	1
Central	4	10	0	0	6	2
South	6	86	3	15	4	7
East	6	1	0	9	1	0
West	7	24	62	181	45	3
Total	28	138	65	243	62	13
Reduction (%)		79.6	91.3	93.0	79.2	82.4

ency of spray programs to reduce parasitism by *Pseudhomalopoda prima* has been observed repeatedly in low to moderate infestations of scale. In two cases it has been possible to study reduction of parasitism in severe infestations. Both experiments included two replications of sulfur, Aramite,® 2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite, and unsprayed test plots. Table 9 presents the data collected from these experiments. Both Aramite and sulfur reduced parasitism by *Pseudhomalopoda prima* with a reflected increase in living scale and a decrease percentage in dead scale. Data from a second series of samples collected from one expriment 3 weeks after the first indicate that the influence of these insecticides on parasitism is prolonged. It is interesting to note, however, that parasitism apparently reduced scale infestations in all plots despite the reduced rate of attack. The combined parasitism of second-stage scale by *Prospaltella aurantii*, *Aspidiotiphagus lounsburyi* and *Aphytis chrysomphali* was not strikingly affected by the chemical treatments.

These studies of factors influencing parasitism indicate that sunlight, rain, low temperatures, and insecticides all will reduce parasitism. Low temperatures and insecticides appear to be most critical, with low temperatures seeming to have the greatest influence. *Pseudhomalopoda prima* is the most sensitive species of parasite and increases in Florida red scale infestations are commonly associated with decreases of this species.

Table 8.—Comparison of % parasitism of third-stage Florida red scale in adjacent sprayed and unsprayed blocks of Marsh seedless grapefruit over a 4-year period.

YEAR	PER CENT PARASITIZED THIRD STAGE SCALES	
	Sprayed Block	Unsprayed Block
1954	4.8	4.7
1955	8.2	25.3
1056	16.1	18.3
1957	13.7	30.2
Average	10.7	19.6

Table 9.—Comparison of live, dead, and parasitized Florida red scale on 40 heavily infested leaves from control, Aramite, and sulfur-treated plots in two experiments at Lake Alfred, Florida. 1955.

TREATMENT	TOTAL SCALE		DEAD SCALE (%)	PARASITIZED SCALE (%)	
	Live	Dead		Second Stage	Third Stage
	Crawford Grove, October 5				
Control	1003	756	42.4	49.0	58.0
Aramite	3746	1963	34.4	57.3	25.4
Sulfur	5072	1473	22.5	66.0	12.3
	October 25				
Control	299	473	61.3	69.7	25.9
Aramite	1387	629	45.3	66.2	18.9
Sulfur	2869	738	25.7	69.8	6.9
	Blocks XV and XVI, October 13				
Control	507	527	50.9	16.3	13.5
Aramite	1563	694	30.7	31.1	4.7
Sulfur	2186	819	27.3	43.6	8.7

REFERENCES CITED

Fisher, Fran E. 1950. Two new species of *Hirsutella* Patouillard. Mycologia, **42**(2), 290–7.

Fisher, Fran. E. 1950A. Entomogenous fungi attacking scale insects and rust mites on citrus in Florida. Jour. Econ. Ent. **43**(3): 305–9.

Fisher, Fran E., W. L. Thomson, and J. T. Griffiths, Jr. 1949. Progress report on the fungus diseases of scale insects attacking citrus in Florida. Florida Ent. **32**(1): 2–11.

Griffiths, J. T., Jr., and C. R. Stearns. 1947. A further account of the effects of DDT when used on citrus trees in Florida. Florida Ent. **30**(1)2): 1–8.

Griffiths, James T., Jr., and W. L. Thompson. 1947. The use of DDT on citrus trees in Florida. Jour. Econ. Ent. **40**(3): 386–8.

Griffiths, James T., Jr., and W. L. Thompson. 1948. A preliminary report of the possibilities of forecasting periods of oviposition activity for purple and Florida red scales. Proc. Florida State Hort. Soc. **61**: 101–10.

Griffiths, James T., Jr., and W. L. Thompson. 1949. Identification of Florida red and purple scales on citrus trees in Florida. Florida Agric. Expt. Sta. Circ. **S5**, 1–13.

Griffiths, James T., Jr., and W. L. Thompson. 1949A. Purple scale and Florida red scale as insect pests of citrus in Florida. Florida Agric. Expt. Sta. Bull. **462**: 1–39.

Mathis, W. 1941. Notes on the biology of Florida red scale. Florida Ent. **24**(1): 1–5.

Mathis, W. 1947. Biology of the Florida red scale in Florida. Florida Ent. **29**(2, 3): 13–35.

Muma, Martin H. 1955. Phytoseiidae (Acarina) associated with citrus in Florida. Ann. Ent. Soc. America **48**(4): 262–72.

Muma, Martin H. 1955A. Factors contributing to the natural control of citrus insects and mites in Florida. Jour. Econ. Ent. **48**(4): 432–8.

Muma, Martin H. 1955B. Lady beetles found on citrus in Florida. Florida Ent. **38**(3): 117–24.

Muma, Martin H. 1955C. Some ecological studies on the twice-stabbed lady beetle, *Chilocorus stigma* (Say). Ann. Ent. Soc. America **48**(6): 493–8.

Muma, Martin H. 1956. Life cycles of four species of lady beetles. Florida Ent. **39**(3): 115–18.

Muma, Martin H. 1957. Effects of larval nutrition on the life cycle, size, coloration, and longevity of *Chrysopa lateralis* Guer. Florida Ent. **40**(1): 5–9.

Muma, Martin H. 1959. Chrysopidae associated with citrus in Florida. Florida Ent. **41**(4): 42(1): 21-29.

Pratt, Robert M. 1952. Seasonal and geographical distribution of some citrus insects and mites in Florida. Proc. Florida State Hort. Soc. **65**: 50–55.

Watson, J. R. 1918. Insects of a citrus grove. Florida Agric. Expt. Sta. Bull. **143**: 166–267.

Watson, J. R., and E. W. Berger. 1926. Citrus insects and their control. Florida Agric. Expt. Sta. Bull. **183**: 291–423.

The Japanese Weevil *Pseudocneorhinus bifasciatus* Roelofs[1]

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ABSTRACT

New information was obtained on the biology of the weevil (*Pseudocneorhinus bifasciatus* Roelofs) infesting ornamentals in New Jersey in 1956 and 1957. The insect hibernated in the adult and egg stages in debris on the ground and as young larvae in the soil beneath infested shrubs. The overwintering weevils resumed feeding on the first hot day of spring, and continued to feed and oviposit throughout the summer. The larvae hatching from these eggs were at most only half grown by the end of August. The eggs deposited in September and October started hatching in the fall and completed hatching in early spring. The larvae developed in the soil beneath infested plants, feeding on their roots. Pupation in this midsummer brood began in early June, and the peak of emergence occurred from early to mid-July.

Larvae, prepupae, and pupae were found only in the soil beneath infested plants, and occurred to depths of at least 9 inches. As many as 150 larvae per square foot, and 51 weevils per yard of privet hedge, were found. Chlordane at 10 pounds per acre in the soil beneath infested plants was an ineffective control.

The Japanese weevil *Pseudocneorhinus bifasciatus* Roelofs attacks many ornamentals in the Eastern States. Life-history data gathered in the spring and summer of 1956 (Allen 1957) are supplmented in this paper with information obtained at Moorestown, New Jersey, from the fall of 1956 to the fall of 1957.

SEASONAL HISTORY.—*Hibernation.*—Adult weevils were abundant on foliage in September 1956, but by mid-October nearly all had left it and entered hibernation. Oviposition was heavy in September and early October, but ceased with the coming of cold weather. Oviposition also stopped by November in a stock of weevils maintained at room temperatures. Some of the eggs deposited in the fall hatched before winter. Thus the insect hibernated in the egg, larval, and adult stages.

Hibernating adults passed the winter in debris under infested plants. In late winter and early spring 24 were found in an 8-square-foot and 18 in a 5-square-foot area, both under infested privet. Hibernating weevils remained sluggish and inactive during the first warm days of spring, but on the first hot day, April 21, they climbed the shrubs above their hibernating quarters and resumed feeding. Moderate numbers of overwintering weevils continued to feed on host plants until long after the spectacular upsurge of adult population resulting from mid-summer emergence. Hibernating weevils were readily distinguished from those freshly emerged by their duller markings and the presence of mature ovarian eggs. During late spring and early summer defoliation was scarcely noticeable, since the effect was masked by the rapidly growing foliage.

Eggs and Larvae.—Forty-two hibernating weevils collected in late winter and early spring were maintained in a cage at normal temperatures. Fresh privet foliage was provided as food, and the egg pods were removed at about weekly intervals. The weevils fed heavily throughout the summer. On August 28, when the experiment was terminated, there were still 12 active survivors. These weevils has produced eggs in abundance since mid-May. A total of 333 egg pods was counted, and some more were produced. The average number of eggs per pod from these caged weevils was 2.6. In May and June hatching usually occurred 14 to 18 days from oviposition. Frequently 1 or 2 days elapsed between the hatching of the

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[2] Assisted by E. L. Plasket.

first and last eggs in a pod. Larval mandibles became visible 2 days and the well-developed head 1 day before hatching.

Early in June, when newly hatched larvae originating from the hibernating weevils were most abundant, several potted privet and strawberry plants were stocked with young larvae and bedded outdoors. Newly hatched weevil larvae dropped on the surface of the soil in the pots and promptly burrowed downward. The soil and roots were examined from time to time. The larvae developed slowly. At the final observation on August 29th, 22 larva were found which were apparently healthy and well nourished, but they were only half grown, being 3.0 to 4.5 mm. long.

Some of the eggs laid by the summer brood in late summer and early fall of 1956 hatched on warm fall days, and probably some even on the milder winter days. Several lots of the eggs were exposed to outdoor weather in jars nested in leaf mulch on the ground. In one lot laid in September and examined on October 22nd, 18 had hatched, 14 had well-developed embryos, and in 5 the embryos were not visible. On the same date there had been no hatching in another lot deposited on October 1 to 3. By December 30 there had been considerable hatching even in eggs deposited as late as October 9, but there were also some viable eggs containing healthy embryos. On March 29 it was found that, although most of the eggs had hatched, a small portion of the eggs deposited in October had overwintered successfully. One hatched to a vigorous larva while the egg was under observation.

Attempts to locate larvae in natural infestations during the fall of 1956 were not entirely successful. However, when newly hatched larvae were placed on the soil in pots containing sections of lilac roots and rootlets, they entered the soil at once, and after a few days they could be found in the vicinity of the root sections, as well-fed young larvae.

Thus it was evident that part of the weevil population entered the winter as eggs in surface debris under infested plants and as young larvae in the soil. The study of this portion of the population was resumed in the spring of 1957. In late April, when hibernating weevils had resumed their feeding, the larvae that had hatched during the previous late summer and fall had developed in the soil beneath infested shrubs to half-grown dingy yellowish to reddish white grubs, 2.5 to 6.0 mm. long.

These grubs were found in abundance under heavily infested privet hedges, some samples having 150 per square foot. In three sample diggings between May 11 and 22, grubs were found to be present from 1 inch to at least 9 inches below the soil surface, with the greatest numbers from 3 to 6 inches below. Soil samples, each of 230 cubic inches, were taken at various distances from the stems at the center of an infested privet hedge. At 1 foot from the center, in an area completely overhung and densely shaded by the hedge, and where privet roots were densely interlaced, there were 17 weevil larvae. Two feet from the plant stem, and at the edge of the hedge, where grass roots and privet roots were intertwined, there were 12 larvae. At 3 feet from the stem, in lawn adjacent to the hedge and where grass roots predominated, there were no weevil larvae. In this observation weevil larvae were sharply restricted to the soil under infested shrubs.

Pupation.—By May 5 some larvae of the midsummer brood had completed their feeding. They were entirely opaque white without discolorations owing to the presence of food in the midgut. By June 3 more than half the larvae had become prepupae, and on that date the first pupa was observed. These also were entirely white. Pupation occurred at various soil depths within the limits inhabited by the larvae. An attempt was made to observe the duration of the prepupal and pupal periods. Unfortunately, when larvae were isolated in moist soil in small vials, so that they could be observed through the glass walls, there was a high mortality. During late May and June the prepupal period for two individuals was 6 days, but for others it was longer. The duration of the pupal period for the one specimen observed was 14 days.

Abundance.—During the spring months surveys of weevil populations on privet hedges were made from time to time by beating half a section of hedge after a cloth sheet had been placed on the ground beneath it, and counting the weevils that dropped on the sheet. The population of hibernated weevils rarely exceeded two per yard of hedge. On June 25, in a very sunny location, the first newly emerged weevils were observed. By July 8 newly emerged weevils greatly exceeded overwintering ones in all locations. On July 15 there were 51 weevils per yard of hedge where there had been about two a month earlier. This abrupt increase in the weevil population was accompanied by a sharp increase in the foliage feeding. Defoliation became progressively more evident as the growth rate of leaf tissue slowed down, and the effects of many weeks of continued feeding accumulated.

Only scanty observations were made on the feeding habits of the larvae. It seems probable that they subsist on the roots of plants on which the adults occur as foliage feeders. The small roots under infested lilac and privet have been observed to have surface wounds which may have been caused by the larvae. Dissection of several larvae of the midsummer brood obtained from the soil under privet revealed that their guts were packed with small pellets of plant tissue that had a cellular structure similar to the bark of privet roots. Normal, well-fed larvae have been reared or dug up where the roots of only privet, forsythia, lilac, or strawberry were available.

CONTROL.—An attempt was made on two heavily infested properties to control the weevil by soil treatments. Chlordane, at the rate of 10 pounds per acre, was applied in October as a coarse spray under all shrubs in the area treated. It was washed into the soil by a liberal wetting with a garden hose. The midsummer emergence of weevils on some of the shrubs in this area was much less than it had been the previous summer, but was as high on others. Some sections of treated privet hedge had as many weevils as nearby untreated sections. The treatment obviously was not effective.

REFERENCE CITED

Allen, H. W. 1957. A Japanese weevil abundant in the Philadelphia area. Ent. News 68: 169–74.

Dorsal Contact Toxicity of Six Insecticides to Wintering Larvae of the European Pine Shoot Moth[1]

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ABSTRACT

Tests were conducted with six organic insecticides in a settling type spray chamber to determine their relative dorsal contact toxicity to overwintering larvae of the European pine shoot moth, *Rhyacionia buoliana* (Schiff.). No significant mortality was obtained with DDT, chlordane, or BHC at any concentration used. Malathion, Thimet® *O,O*-diethyl *S*-(ethylthio) methyl phosphorodithioate, and dimethoate produced mortality at all concentrations used. Failure of DDT to act as a contact insecticide in these tests suggests that the excellent control obtained with it in the field is due to the residue acting as a stomach poison.

This report presents results of a laboratory study in which a settling type spray chamber was used to apply known amounts of six insecticides to larvae of the European pine shoot moth, *Rhyacionia buoliana* (Schiff.). The test insects were wintering (fourth-instar) larvae removed from infested red pine shoots. Following treatment in the spray chamber, the insects were held at a constant temperature and humidity, and counts of their mortality were made at 24-hour intervals for a 96-hour period.

MATERIALS AND EQUIPMENT.—The insecticides tested were DDT (73% and 75% para para isomer), chlordane (100% active ingredient), BHC (47.5% gamma isomer), malathion (95% active ingredient), Thimet® (*O,O*-diethyl *S*-(ethylthio) methyl phosphorodithioate) (90% active ingredient), and dimethoate (90% active ingredient). All insecticides were of technical grade. It was not possible to make any compensation for the insecticidal activity of the secondary isomers of DDT, chlordane, and BHC, so these materials were considered 100% active ingredient. The organic phosphates—malathion, Thimet, and dimethoate—were used in compensated amounts to offset the known inert ingredients in their formulations.

The spray chamber used was a 4-foot-long metal box divided into two compartments. The first was a small spray-tight compartment in which spray was continuously discharged against a shutter mechanism. The shutter covered a $\frac{3}{4}$-inch opening into the larger settling compartment. By means of a timing wheel and solenoid, shutter action could be controlled to within 1/20 second. When the shutter was activated, the spray passed through the open part into the settling compartment and settled on the test insects. The insecticide was propelled by compressed carbon dioxide regulated at 5 pounds per square inch. The atomizer nozzle produced a drop size of about 80 microns mass median diameter (Drooz 1957).

PROCEDURE.—The spray solutions were prepared by dissolving the insecticide in 500 milliliters of xylene. Four and one-half grams of Dupont oil red dye was added for tracer dye determinations in a colorimeter. Enough kerosene was added to bring the solution up to 1892.5 milliliters ($\frac{1}{2}$ gallon). The xylene-kerosene-dye mixture by itself had no apparent effect on larvae during a 96-hour post-treatment observation period (table 1).

The spray chamber was calibrated by producing a dye deposit on a 6-inch square aluminum plate and then washing this plate with 25 milliliters of acetone. The acetone washings were then compared with a dye solution which had been prepared to represent an insecticide concentration of 1 gallon per acre. The average of three colorimeter readings was used to make this comparison. After the spray chamber was calibrated, checks on its output were made before and after each treatment. The control solution used in the colorimeter for determining the rate of application before and after each test was drawn from the same stock solution that was to be used for treating a given group of larvae.

The European pine shoot moth larvae used in these tests were collected from infested red pine trees on the grounds of the Rose Lake Experiment Station, Michigan Department of Conservation, near Lansing. Collections were made from November 1956 to February 1957. The infested tips were stored at 50° F. and 80% relative humidity until they were dissected for larvae. It was possible to keep tips 3 weeks before they became too dry to dissect conveniently.

Because of progressing winter mortality, the shoots collected in February yielded half as many larvae as those collected in November. However, the larvae obtained from the February collections had a higher survival in the checks than those collected earlier (table 2). About 40% larval mortality occurred from mechanical injury in removing the insects from the tips.

After the larvae were removed from the tips, they were placed in a 1,000-milliliter beaker with a ring of clear petroleum jelly around the rim to prevent their escape. When a strong light was placed over the beaker, most of the healthy larvae began to crawl upward. They were then picked from the side of the beaker and placed in pill boxes by means of tweezers which had a safety spacer to prevent injury. If a larva, at the beginning of a treatment, was observed to be sluggish or injured, it was replaced with a healthy one.

The larvae were treated rapidly. A pile of clean 6- by 8-inch sheets of paper was placed on a hard surface in front of the settling chamber. An open pill box with 10 healthy larvae was then inverted and struck sharply downward against a paper. This stunned the insects for about 20 seconds. The paper with the larvae was placed in the settling chamber and the insecticide was allowed to pass into the compartment. This paper was removed after a 10-second spray settling period. Upon removal, the larvae were hanging tenaciously to the paper, but still in their original position. The paper was then grasped at each end, inverted, and snapped over an open pill box.

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[2] Acknowledgment is made to the Department of Entomology, Michigan State University, for counsel and for providing laboratory space.

Table 1.—Mortality comparison of 40 larvae of the European pine shoot moth[a] treated with the xylene-kerosene-dye mixture and 40 untreated larvae.

Cumulative Percentage Mortality After Hours Shown			
24	48	72	96
Untreated			
0	0	3	8
Treated			
0	0	3	3

[a] Collected on February 5 and treated on February 20, 1957.

This caused the larvae to be thrown back into their original container without being directly handled, and without having had time to crawl about on the paper. Therefore, the larvae came in contact only with the insecticide settling on them in the spray chamber. After each run with a particular insecticide, the settling compartment was washed with acetone.

After removal from the spray chamber, the larvae were placed in a cabinet which was maintained at 78° F. (± 5 degrees) and 58% relative humidity (± 10%). The dead and living larvae were counted every 24 hours for 4 days. If a larva was missing, the pill box was examined closely for evidence of cannibalism. If such evidence was present, the destroyed insect was not counted in the test.

Four replications of 10 insects each were treated at each concentration of insecticide. The test for a particular concentration of insecticide and the check test were run on the same day with larvae collected at the same time.

A preliminary test with DDT was run to determine a starting dosage. DDT concentrations of 1, 2, 4, and 8 grams per gallon had no effect on the larvae. Another test at 16 grams per gallon was run, but still without significant larval mortality. To determine whether all the other insecticides in the study required such a high dosage to cause significant mortality, a starting point of 32 grams per gallon was decided upon. Succeeding concentrations were 64 and 128 grams per gallon.

Concentrations of DDT and BHC greater than 128 grams per gallon probably cannot be obtained with the solvent mixture used in this study. Dissolving 128 grams of technical BHC and DDT took about 36 hours, plus the application of heat.

RESULTS.—No significant mortality resulted from any chlorinated hydrocarbon treatments (table 3). This was surprising since DDT has consistently proved to be the best performing material in field tests (Miller & Neiswander 1955, Guyer *et al.* 1957, and others). Although the maximum spray chamber rate of about 0.3 pound per acre (128 grams per gallon) was much less than field application rates, the uniformity and completeness of coverage in the spray chamber can be considered to offset much of the difference between spray chamber concentrations and the usual field dosages.

Table 2.—Mortality in check larvae of the European pine shoot moth treated with the xylene-kerosene-dye mixture on different dates.[a]

Date		Cumulative Percentage Mortality After Hours Shown			
Treated	Collected	24	48	72	96
Jan. 4	Dec. 18	5	13	15	33
Jan. 20	Jan. 5	3	5	8	21
Feb. 3	Jan. 25	5	10	16	22
Feb. 20	Feb. 5	0	0	3	3

[a] Forty larvae were treated on each date (1957).

Table 3.—Results of spray chamber tests on wintering larvae of the European pine shoot moth, 1956–57.

Material	Number[a] of Larvae Used	Cumulative Percentage Mortality After Hours Shown			
		24	48	72	96
16 Grams per Gallon, January 4					
DDT	40	0	0	10	25
Untreated	40	3	5	5	10
32 Grams per Gallon, January 20					
DDT	40	3	3	3	10
Chlordane	40	8	28	33	43
BHC	40	0	20	23	30
Malathion	40	45	73	83	83[b]
Thimet	39	40	70	70	74[b]
Dimethoate	40	45	53	55	58[b]
Untreated	34	3	5	8	21
64 Grams per Gallon, February 3					
DDT	40	0	15	31	33
Chlordane	40	3	8	18	43
BHC	38	3	16	34	47
Malathion	40	70	85	90	93[b]
Thimet	39	50	73	85	95[b]
Dimethoate	40	65	85	90	98[b]
Untreated	39	5	10	18	26
128 Grams per Gallon, February 20					
DDT	40	0	0	3	8
Chlordane	40	0	0	0	8
BHC	40	0	0	0	3
Untreated	39	0	0	3	3

[a] Each test was begun with 40 larvae. The differences from the original 40 larvae in the column were caused by cannibalism.
[b] Significantly different from the check at the 5% level 96 hours after treatment.

The failure of the chlorinated hydrocarbons to bring about significant mortality in these tests show that the dorsal dermal area of the larva was not a main entry point for these materials in xylene-kerosene solvent. Although the permeability of ventral body areas and tarsi has not been investigated, the above results suggest that the chlorinated hydrocarbons may act primarily as stomach poisons in the field.

In contrast to the chlorinated hydrocarbons, the organic phosphates brought about significant mortality (table 3). The effects of the three tested members of this group were alike. At the 64-gram rate, between 85- and 90% mortality resulted with dimethoate, malathion, and and Thimet on the third posttreatment day. There were slightly less mortalities at the 32-gram rate, but they still fell in the upper half of the mortality scale.

Despite good laboratory results, malathion has not performed in the field as well as DDT at the concentrations tested (Miller & Neiswander 1955, Cath 1953). It is inferred here that the field inadequacy of this material was due to its failure to reach the insect. All the larvae in the field are not exposed at any one time, and the material probably dissipates before some of the larvae be-

come exposed. Thimet and dimethoate have not yet been compared with DDT under field conditions.

REFERENCES CITED

Cath, W. S. 1953. Studies on control of the European pine shoot moth, *Rhyacionia buoliana* (Schiffermuller). 61 pp. Unpublished Master's Thesis, Michigan State University.

Drooz, A. T. 1957. Spray chamber insecticide tests on the larch sawfly (*Pristiphora erichsonii* (Htg.)). Canadian Ent. **89**: 183–7.

Guyer, G., W. F. Morofsky, and W. Lemmien. 1957. The evaluation of insecticides for control of the European pine shoot moth under spring and summer conditions. Michigan Agric. Expt. Sta. Quart. Bull. **39**: 432–7.

Miller, W. E., and R. B. Neiswander. 1955. The biology and control of the European pine shoot moth. Ohio Agric. Expt. Sta. Res. Bull. **760**: 1–31.

Control of *Hypera postica* (Gyll.) on Second-Cutting Alfalfa in New York[1]

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ABSTRACT

A total of 15 counties in New York is presently known to be infested by the alfalfa weevil, *Hypera postica* (Gyll.)(Curculionidae: Coleoptera). Larval feeding in 1958 was delayed on the first alfalfa cutting and resulted in injury to the second crop. Five replicated experiments were conducted in second-cutting fields in Dutchess, Orange, and Ulster Counties in which 15 insecticides were applied as low pressure, low volume sprays.

Heptachlor at 4 ounces actual material per acre gave excellent control of larvae for 32 days following application. Chlordane at 32 ounces and lindane and endrin each at 4 ounces per acre gave good larval control for 2 weeks after treatment, while phosphorus-containing compounds gave effective larval control for slightly more than 1 week. Heptachlor applied at a rate of 8 ounces per acre gave good control of the alfalfa weevil adult for 2 weeks following application.

The rapid spread of the alfalfa weevil, *Hypera postica* (Gyll.), in New York has necessitated an extensive investigation of control measures in an attempt to prevent widespread serious forage losses. The alfalfa weevil was first found in New York in Orange County in 1955 (Neunzig *et al.* 1955); a total of 13 counties was known to be infested by 1957. During 1958 the weevil was found 50 miles north of the known 1957 infestation. Extensive alfalfa losses, caused by larval feeding, occurred only in Orange County in 1958. In some fields, first-harvest yield reductions were estimated to approach 50%.

Unseasonably warm temperatures for a short time during the early spring of 1958 in New York caused unusually early alfalfa growth. However, alfalfa weevil activity apparently was not greatly influenced by these conditions, and larval damage did not appear until the first-cutting crop was nearly ready for harvest. As is the practice generally in southern New York, the first harvest was removed for ensilage.

Field observations indicated that alfalfa removed by field choppers resulted in removal from the field of nearly all the larvae present. Eggs remaining in the stubble, probably together with the activity of gravid females, gave rise to larval populations sufficiently large to cause some second-cutting losses. All experimental work reported here was conducted on second-cutting alfalfa.

EXPERIMENTAL PROCEDURE.—The randomized complete block design was used in each of the five experiments; plots were 20 feet by 60 or 75 feet in dimensions. All insecticides were applied as emulsion or similar-type sprays using low-pressure, low-volume apparatus.

At different intervals after application, 30 or 40 sweeps with a 15-inch-diameter collecting net were taken in systematically selected subplots. No subplots were sampled more than once. Insects taken were transferred, using a cellulose acetate funnel, into 8-ounce specimen jars half filled with alcohol for counting at a later date.

Experiment A was replicated three times; 2 replicates were located in Orange County and 1 in Dutchess County. Foliage height measured 2 to 6 inches on the application date. Plots were sampled for larvae 7, 17, and 32 days after treatment (table 1).

Experiment B, situated in Ulster County, was replicated five times. Foliage height averaged 11 inches on the date of application. Larval sampling was conducted 7 and 17 days after application (table 2).

Experiment C was replicated five times and was located in Dutchess County. Foliage height measured 8 to 12 inches on the date of application, after which time larvae were sampled at 5 and 16 days (table 3). Since a large number of adults was present at the 16 day interval, these data are included also.

Experiment D, located in Orange County, was replicated five times. Alfalfa height averaged 4 inches on the date of application. Plots were sampled for larvae 5 and 14 days after treatment (table 4).

Experiment E, conducted entirely for control of adults, was replicated five times and was situated in Ulster County. Foliage height, on the application date, ranged from 12 to 15 inches. Plots were sampled for adults 6 and 13 days after insecticidal treatment (table 5). All sweeps were taken at night in order to obtain maximum numbers of adults (Essig & Michelbacher 1933).

Statistical analyses of all data were conducted using analysis of variance. Insect counts were first transformed: $X' = \sqrt{X + 0.5}$, where X is the total number of insects

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[2] The authors acknowledge assistance given by Donald W. Adams.

Table 1.—Evaluation of low-pressure, low-volume sprays for effectiveness in controlling the larva of the alfalfa weevil. 1958.

Material[a]	Actual Toxicant per Acre (Oz.)	Average No. Larvae per 30 Sweeps[b]	Percentage Control
	7-day sample		
Heptachlor	4	0.0	100
Heptachlor	8	0.0	100
Malathion	8	0.0	100
Heptachlor	6	0.3	98
Methoxychlor	16	1.3	91
Endrin	4	2.0	87
Methoxychlor	32	3.7	76
Malathion	4	15.3	0
Untreated	—	15.3	0
	17-day sample		
Heptachlor	8	3.0	97
Heptachlor	6	5.3	94
Heptachlor	4	7.0	92
Endrin	4	26.2	71
Methoxychlor	16	49.3	45
Methoxychlor	32	47.7	47
Malathion	8	45.0	50
Malathion	4	91.4	0
Untreated	—	90.3	0
	32-day sample		
Heptachlor	6	0.3	96
Heptachlor	8	0.3	96
Heptachlor	4	0.3	96
Malathion	4	4.3	48
Methoxychlor	32	5.7	32
Malathion	8	6.4	24
Methoxychlor	16	7.7	8
Endrin	4	10.0	0
Untreated	—	8.3	0

[a] Treatments applied June 13 and 14. Sampled June 20 and 21, June 30 and July 1, and July 15 and 16. All emulsifiable concentrates.
[b] Any two means flanked by the same line are not significantly different at the 5% level.

taken in the sample from each subplot. Duncan's multiple range test, at the 5% level, was employed to test for significant differences among means.

RESULTS.—Experiment A—Heptachlor applied at rates of 4, 6, or 8 ounces and malathion at 8 ounces per acre gave excellent control of the larva of the alfalfa weevil 8 days after application (table 1). Endrin at 4 ounces and methoxychlor at 16 or 32 ounces per acre also gave good larval control. Malathion applied at 4 ounces per acre produced no noticeable effects on larval populations.

At the 17-day interval heptachlor applied at either 4, 6, or 8 ounces per acre continued to provide excellent control of larvae. Endrin produced substantial larval reductions at this time while all the other materials used gave various degrees of poor control. When the plots were sampled 32 days after treatment, heptachlor at all dosages used gave excellent control; all other compounds used afforded poor larval control.

Experiment B—Seven days after application, lindane at 6.4 ounces per acre gave excellent reductions of larval numbers (table 2). Ethion at 16 ounces, and Trithion® (*S*-(p-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiate) each applied at 8 ounces per acre provided only fair control, while Dibrom®(*O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate) at 16 ounces per acre was ineffective.

Table 2.—Effectiveness of low pressure sprays in controlling the larva of the alfalfa weevil. 1958.

Material[a]	Actual Toxicant per Acre (Oz.)	Average No. Larvae per 40 Sweeps[b]		Percentage Control	
		7 Days	17 Days	7 Days	17 Days
Lindane	6.4	5.2	4.8	91	73
Trithion	8	17.2	16.2	71	9
Ethion	16	17.4	15.2	71	15
Diazinon	8	20.2	19.6	66	0
Dibrom	16	31.2	19.2	48	0
Untreated	—	59.8	17.8	0	0

[a] Treatment applied June 16. Sampled June 23 and July 3. All emulsifiable concentrates except Trithion, which was flowable powder.
[b] Same as b, table 1.

At the 17-day interval lindane had decreased somewhat in effectiveness as compared with the 7-day results, while all the other materials used gave poor or no larval control.

Experiment C—Sampling data taken 5 days after application showed that all the phosphorus-containing compounds used, viz., Guthion® (*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate), parathion, and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical), compared favorably with heptachlor for effectiveness in larval control (table 3). Each gave excellent control. Eleven days later heptachlor continued to provide excellent control, while the other compounds used gave no control.

Heptachlor gave excellent control of adults of the alfalfa weevil at the 16-day interval, while the phosphorus-containing insecticides used gave various degrees of poor control. This undoubtedly is a reflection of the poor larval control afforded by the latter compounds.

Experiment D—When plots were sampled at the 5-day interval, methoxychlor at 16 ounces per acre gave excellent larval control, while the use of Diazinon at 16 ounces, chlordane at 32 ounces, and lindane at 4 ounces resulted in good control (table 4). Although heptachlor at 8 ounces per acre appeared to provide poor control, variability in the larval counts was extremely high, and no significant differences were detected among any of the insecticidal treatments.

At the 14-day interval the use of heptachlor, chlordane,

Table 3.—Evaluation of low-pressure sprays for effectiveness in controlling the larva and adult of the alfalfa weevil. 1958.

Material[a]	Actual Toxicant per Acre (Oz.)	Average No. Insects per 40 Sweeps[b]			Percentage Control		
		Larvae		Adults	Larvae		Adults
		5 Days	16 Days	16 Days	5 Days	16 Days	16 Days
Heptachlor	8	8.8	0.2	0.4	93	99	99
Guthion	4	5.2	28.0	31.2	96	0	50
Parathion	8	5.6	26.2	29.8	96	0	53
Phosdrin	8	8.6	39.6	45.0	93	0	28
Guthion	8	8.6	31.4	31.0	93	0	51
Untreated	–	127.3	24.6	62.8	0	0	0

[a] Treatments applied June 18. Sampled June 23 and July 4. All emulsifiable concentrates.
[b] Same as b, table 1.

Table 4.—Effectiveness of low-pressure sprays in controlling the larva of the alfalfa weevil. 1958.

Material[a]	Actual Toxicant per Acre (Oz.)	Average No. Larvae per 30 Sweeps[b]		Percentage Control	
		5 Days	14 Days	5 Days	14 Days
Methoxychlor	16	3.0	96.9	94	56
Diazinon	16	6.0	108.5	89	51
Chlordane	32	7.4	18.4	86	92
Lindane	4	12.2	45.4	77	80
Heptachlor	8	25.6	32.4	51	85
Untreated	—	52.2	221.0	0	0

[a] Treatments applied June 19. Sampled June 24 and July 3. All emulsifiable concentrates.
[b] Same as b, table 1.

Table 5.—Evaluation of low pressure sprays for effectiveness in controlling the adult of the alfalfa weevil. 1958.

Material[a]	Actual Toxicant per Acre	Average no. Adults per 40 Sweeps[b]		Percentage Control	
		6 Days	13 Days	6 Days	13 Days
Heptachlor	8	2.8	3.8	82	70
Heptachlor	4	4.8	5.0	68	61
Thiodan	8	13.0	8.2	15	36
Thiodan	4	10.4	9.2	32	28
Dylox	8	13.0	11.2	15	16
Untreated check	—	15.2	12.8	0	0

[a] Treatments applied July 19. Sampled July 25 and August 1. Heptachlor was emulsifiable concentrate, Thiodan was miscible, and Dylox was soluble powder.
[b] Same as b, table 1.

and lindane resulted in large reductions in larval numbers, while methoxychlor and Diazinon gave poor control.

Experiment E—Heptachlor at 8 ounces gave good adult control 6 days after application, while the same material applied at 4 ounces per acre was somewhat less effective. Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) at 4 or 8 ounces, and Dylox®(*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) at 8 ounces per acre gave poor control. Thirteen days after treatment, heptachlor at 8 or 4 ounces per acre provided less effective control than at the 6-day interval, while the other materials used gave various degrees of poor adult control.

CONCLUSIONS.—With larval damage delayed on the first alfalfa cutting in 1958, there was a need for an insecticide that would persist for a long time in its toxicity on the second-cutting. Materials having a long residual action, providing unlawful residues do not exist at harvest, should also be more useful in preventing losses on the first-cutting than those with limited residual effectiveness. Heptachlor, applied at rates as low as 4 ounces of actual toxicant per acre, provided excellent larval control for periods up to 32 days. Materials which gave good larval control for a period of about 2 weeks included chlordane at 32 ounces, lindane at rates as low as 4 ounces, and endrin at 4 ounces per acre. None of the phosphorus-containing insecticides used afforded effective larval control for much longer than 1 week. Heptachlor applied at a rate of 8 ounces per acre gave good control of the adult of the alfalfa weevil up to 2 weeks following application.

REFERENCES CITED

Neunzig, H. H., C. S. Koehler, and George C. Gyrisco. 1955. The alfalfa weevil in New York. Ann. Rept. Ent. Soc. Ontario **86**: 103.

Essig, E. O., and A. E. Michelbacher. 1933. The alfalfa weevil. California Agric. Expt. Sta. Bull. **567**, 99 pp., illus.

Comparative Spray Coverage of Cotton Plants with a Conventional and a High-Clearance Sprayer[1]

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ABSTRACT

Both conventional and high-clearance sprayers placed the heaviest concentration of spray on parts of the plants where there were the most boll weevils (*Anthonomus grandis* Boh.). The conventional sprayer gave better coverage on the interior of the plant and on the under sides of the plant parts, but because of its low clearance, plants in tall cotton were damaged and the injury offset its superiority in spray placement.

One of the questions constantly confronting entomologists in the application of insecticides is whether the spray or dust is reaching an effective site on the plant. Literature on the subject is voluminous. Much of the pertinent information is found in the reviews of Brown (1951) and Brann (1956). Distribution from aircraft has been discussed by Chamberlin *et al.* (1955), and the problem of small-plot spraying by Yoemans & Poos (1955).

Most of the insecticides recommended for the control of the boll weevil (*Anthonomus grandis* Boh.) have a short residual action. Therefore, to gain the most effective control the insecticide should be placed on the plant so that the insect will come in contact with it as soon as

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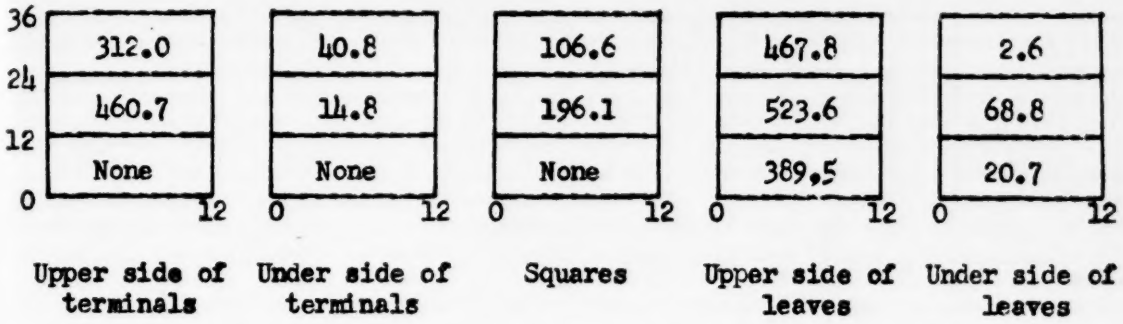

Fig. 1.—Spray distribution with a conventional sprayer, first test, June 20. Number of droplets per square inch.

possible. To determine whether the sprayers utilized in field testing of insecticides at the Florence, South Carolina, laboratory were meeting this requirement, tests were conducted with a conventional and a high-clearance sprayer during the 1957 growing season.

PROCEDURE.—Paper leaves were cut from graph paper with actual cotton leaves as silhouettes. These leaves were made in four sizes representing the range found on the field plants. Graph paper was chosen because the spray droplets had been found to spread on its surface approximately as on a cotton leaf. Paper squares were also cut and folded to simulate natural cotton squares. Circles of the same paper 1¼ inch in diameter were cut to cover the plant terminals.

These simulated plant parts were attached to the plants at random with insect pins. Each leaf was pinned by cutting it from the petiole and then inserting the pin, which had been run through the simulated paper leaf, down into the end of the severed petiole. Paper leaves of the sizes nearest to those of the leaves cut off were used, and the leaves were kept as much as possible in normal position on the plant. In a similar manner paper squares were substituted for cotton squares on the plant. Pins were inserted through the center of the paper circles and run down into the natural terminal so that they were securely fastened. All simulated plant parts were numbered.

The sprays were applied with commercial sprayers of the type in general use throughout the Cotton Belt. The spray booms were adjusted to heights commonly used in experimental and commercial spraying, and had three 3× cone (TeeJet) nozzles per row. One test was conducted on June 20 with a conventional sprayer alone and another on July 18 with both a conventional and a high-clearance sprayer.

An endrin emulsion was applied at the rate of 5 gallons per acre. One package of cardinal red dye and 1 quart of red drawing ink were included in each 5 gallons. When the spray had dried, the simulated plant parts were removed, and the height above the ground and the lateral distance from the center stalk recorded for each part to locate them in the various plant sectors.

Droplets were counted on the following numbers of sample areas: squares 4; upper and under surfaces of small, medium, large, and extra large leaves 3, 4, 6, and 8, respectively; and upper and under terminal surfaces 2 each. From these counts the numbers of droplets per square inch were computed and their distribution determined.

RESULTS.—The distribution of the spray droplets and also of the squares, weevil-punctured squares, and adults on the plants found in the first test are shown in figures 1 and 2. Similar data obtained in the second test are shown in figures 3 and 4. The numbers on the vertical axes indicate inches above the ground, and those on the horizontal axes inches from the central stalk.

Figure 2 shows that the distribution of the adult weevils on the plants was approximately the same as that of the squares. This is indicated by the position not only of the adults but also of the punctured squares. Therefore, the most satisfactory spray distribution would place the heaviest concentration in the vicinity of the largest number of squares. Figure 1 shows that distribution of the droplets on the squares was the reverse of the desired placement; however, the droplets reaching the squares were fairly numerous. The leaves in the vicinity of the squares showed many more droplets per square square inch than the squares themselves. This would be expected, since the leaves of a cotton plant protect the squares behind them from an outward and upward direction at approximately the angle at which the conventional sprayer nozzles are directed into the plant. The under side of terminals and leaves received very little spray. The behavior pattern of the boll weevil on the cotton plant is not completely known. However, if the weevil spends any appreciable time on the under side of the terminals and leaves, its chance of exposure to a fatal dose of insecticide is decreased.

Figure 4 shows that the weevil distribution in the samples taken on July 1 and 19 was similar to that of the squares and bolls on the plants. The punctures

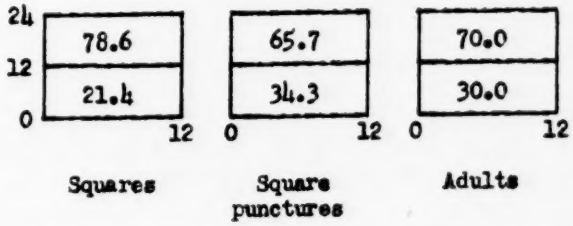

FIG. 2.—Distribution of squares, square punctures, and adult weevils on plants sprayed on June 20. Per cent of total found in samples.

also show the same distribution as the squares, but a tendency for more weevil activity on the younger bolls. These facts indicate the necessity of placing the sprays in the area of the largest number of squares and bolls. Figures 3 and 4 indicate that the highest concentration of spray droplets occurs in the same areas as the largest numbers of punctures—a reliable criterion of adult activity.

The conventional sprayer appears to be slightly superior in that the squares received more spray and there was less interception of the spray by the leaves. Evidently the horizontal arrangment of the high-clearance sprayer places the side nozzles at a less effective angle so that there is greater interception by the leaves and fewer droplets reach the squares behind them.

Neither sprayer placed any appreciable quantity of spray on the under side of the leaves, but the conventional low-clearance sprayer seemed to be more effective in this respect. The exposure of the under side of the leaves as the tractor passed over the plants permitted more droplets to reach these surfaces. However, this desirable disturbance may be offset by its damage to maturing bolls.

Both sprayers placed adequate amounts of spray on

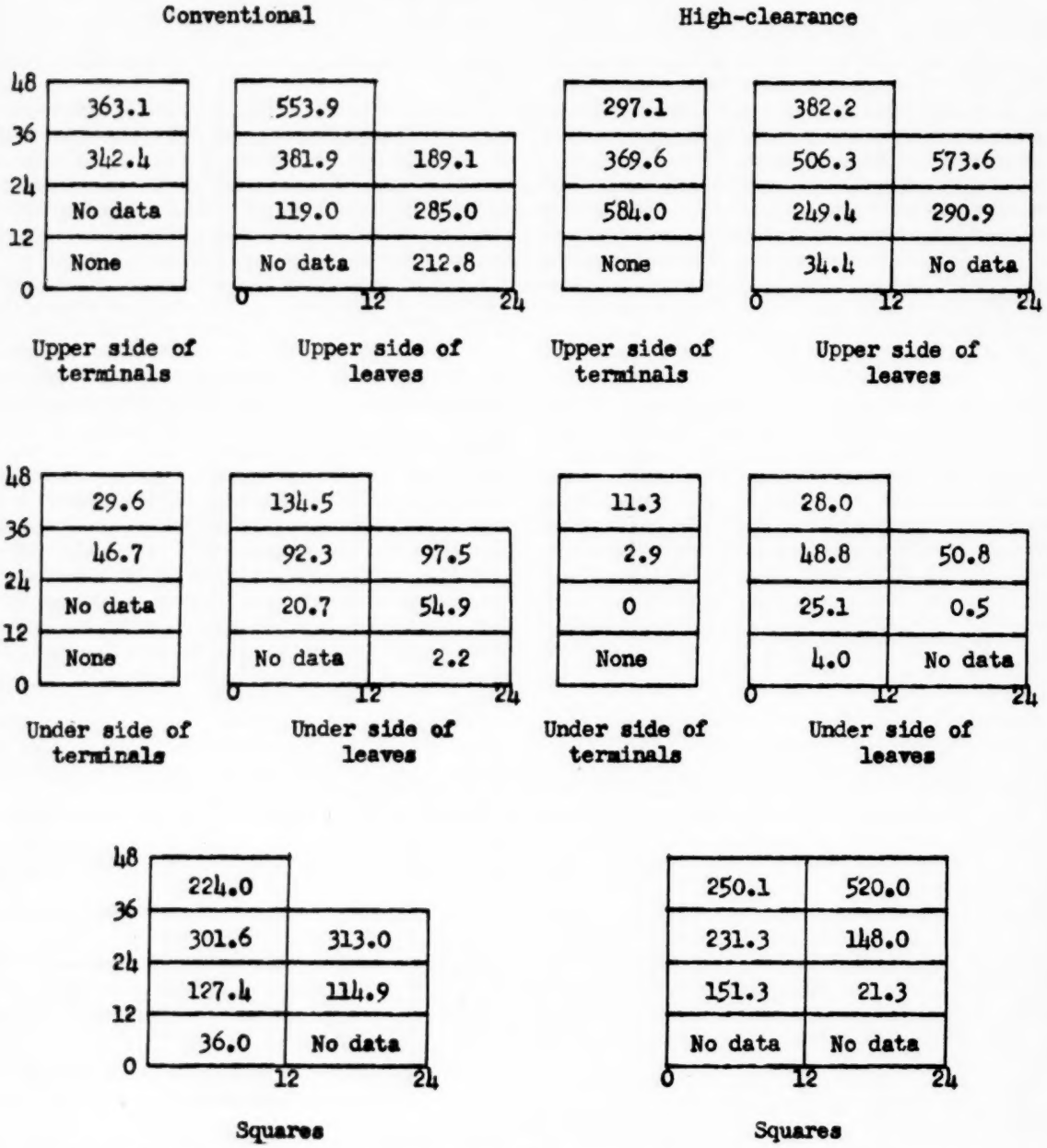

FIG. 3.—Spray distribution with a conventional and a high-clearance sprayer, second test, July 18. Number of droplets per square inch.

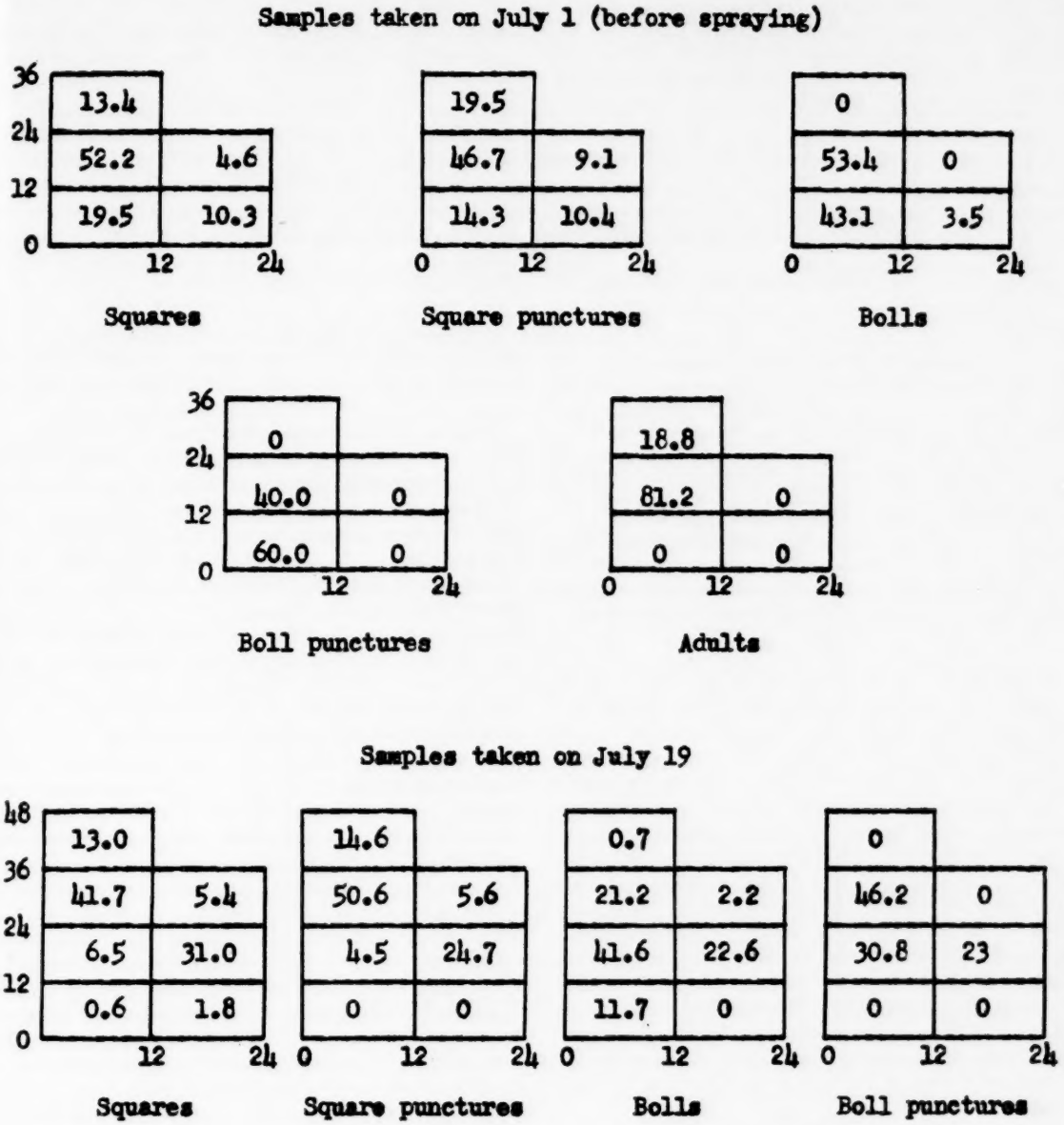

FIG. 4.—Distribution of squares, bolls, punctures, and adult weevils on plants sprayed on July 18. Per cent of total found in samples.

the upper side of the terminals, but very little on the under side. The disturbance of the leaves by the conventional sprayer also gives better coverage on the under side of the terminals, but the severe treatment received by these growing terminals again offsets this advantage.

With both sprayers interception of spray by the outer leaves caused a marked decrease in the amount reaching the inner portion of the plant. When leaves overlapped, the upper leaf drastically reduced the amount of spray reaching one under it. Frequently on the paper leaves the pattern of the upper leaf could be distinctly seen in the lower leaf as an outline of an area with fewer of the colored droplets.

These tests show that both sprayers place the heaviest concencentration of spray in the proper location to reach the greatest number of weevils. However, there is a need for improvement in the placing of spray on the inner portions of the plant, which may harbor sufficient weevils to raise the infestation to injurious levels.

The data indicate that neither sprayer could be considered superior to the other. Considerable damage to maturing cotton by the conventional sprayer offset its superiority in spray placement into the inner portion of the plant.

REFERENCES CITED

Brann, J. L., Jr. 1956. Apparatus for application of insecticide. Ann. Rev. Ent. 1: 241–60.

Brown, A. W. A. 1951. Insect control by chemicals, Chapt. 5, 337–413. John Wiley and Sons, New York.

Chamberlin, J. C., C. W. Getzendaner, H. W. Hessig, and V. D. Young. 1955. Studies of airplane spray deposit patterns and low flight levels. U. S. Dept. Agric. Tech. Bull. 1110. 45 pp.

Yoemans, A. H., and F. W. Poos. 1955. Spray distribution with boom sprayers. U. S. Dept. Agric. ARS 33-8. 7 pp.

Biological Control of *Saissetia nigra* (Nietn.) in California[1]

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ABSTRACT

In California during the first half of the present century, the nigra scale, *Saissetia nigra* (Nietn.), a tropical insect, adapted itself to subtropical conditions and increased to very destructive numbers on various ornamental plants and native vegetation. The maximum population density occurred in 1939. The upward trend was then drastically reversed by the action of the hymenopterous endoparasite *Metaphycus helvolus* (Comp.), which had been colonized on nigra scale infestations in 1938. Within a few years the nigra scale population in California had fallen to a completely noneconomic density. The continual action of *M. helvolus* populations produced on associated scale insects against the nigra scale tends toward the eradication of the nigra scale.

The spectacular increase and decrease in California of the nigra scale, *Saissetia nigra* (Nietn.), during the late 1930's and early 1940's was a phenomenon of special ecological and economic interest. Prior to its upsurge in great numbers along the California coast in the 1930's, this scale insect was supposedly limited to tropical environments. In California, however, it proved to be well adapted to subtropical conditions, arid as well as humid. In spite of the unusually cold winter of 1936–37 in California, the nigra scale by 1938 had become the most common lecanine scale infesting woody ornamental plants from San Diego in the south to Santa Rosa in the north (Smith 1944). In 1938, however, a remarkable reversal in the population trend began. This resulted from the introduction of the endoparasitic wasp *Metaphycus helvolus* (Comp.) into the habitats of the nigra scale by the Department of Biological Control of the University of California.

The rise and fall of the nigra scale as a pest of ornamentals in California is discussed below.

THE UPSURGE OF NIGRA SCALE.—According to published records the nigra scale was first observed in California outside of greenhouses during 1920, it then being found infesting *Euonymus* on the campus of Stanford University (Ferris 1920). In 1931 a large *Pittosporum* on this campus was observed to be heavily infested. It was then prognosticated (Essig 1931) that this scale might in the future prove to be of economic importance, although it was considered unlikely to rival the black scale, *Saissetia oleae* (Bern.), which had become "the most important injurious insect in the state." In less than 5 years, however, the nigra scale had become a pest of major importance to California nurserymen and was of considerable concern to home owners who possessed one or more of the hundred different species of trees and shrubs that were subject to its attack. Many of these host plants appeared black with the nigra scale and the "sooty-mold" growing on the scale's excrement. In towns and cities, sidewalks and cars parked under infested plants became sticky with the honeydew therefrom (Smith 1944).

Prior to 1940 numerous experiments by entomologists of the University of California and the practical experience of commercial spray operators had shown that the nigra scale was more difficult to control by insecticides than any other lecanine scale commonly infesting ornamental trees and shrubs (Smith 1944). This difficulty resulted in part from the resistance of adult nigra scale to chemical treatments. Nurserymen are quoted as saying that "the oil spray just bounced on the scales' backs, but did not kill them" (Agricultural Commissioner, Santa Clara County, *in litt.*). The ability of the female nigra scale to deposit hundred of eggs for an exceptionally long period increased the difficulty of controlling this scale by chemical applications. The oviposition period of a single female ranges from 4 to 10 months. Each egg is a potential female, thus no energy is wasted in the production of males. Although the nigra scale has only one generation annually, it is possible for a population to include individuals in all stages of development at any one time.

The eggs hatch from 1 to 3 weeks after their deposition. The developmental rate of the larvae depends on seasonal conditions, however, and varies greatly. Regardless of their age, nearly all the larvae attain maturity in April, May, or June. Hatching of the eggs is usually completed by midwinter. In cool, well-shaded habitats, and when not subjected to parasitization, the scales may be found in all stages of development (Smith 1944).

Smith (1940) had concluded that it remained to be seen whether the nigra scale would have cycles of abundance in given areas or would remain at a more or less uniform status of abundance throughout successive years.

THE BIOLOGICAL CONTROL PROGRAM.—Fortunately, the oviposition habit of the nigra scale that rendered it difficult to control by insecticides made it particularly vulnerable to attack by natural enemies. This was recognized by Smith (1944), who stated that the nigra scale, because of its egg-laying habits, supports on a single generation several generations of the egg-feeder *Scutellista cyanea* Mots., whereas but one generation normally develops on a given generation of any other species of scale.

The mounting importance of controlling the nigra scale, as revealed by its attack on ornamentals during the late 1930's, culminated in a thorough and exhaustive study of its biology by Smith (1944). The publication of

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this excellent work was, in a sense, anticlimactic because of prior elimination of the nigra scale as a pest of ornamentals through the action of *Metaphycus helvolus*.

The Department of Biological Control first undertook control of nigra scale by natural enemies in the fall of 1936. As stated by H. S. Smith in an annual report for 1939 to the U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine, concerning importations into California of foreign natural enemies, the increase in importance of nigra scale as a pest of ornamentals made the importation of its natural enemies desirable. In a letter to D. T. Fullaway of the Hawaiian Territorial Board of Agriculture and Forestry, dated September 5, 1936, the writer pointed out this need and requested shipments of parasitized nigra scale. As a result of Fullaway's cooperation, three shipments of parasitized nigra scale were received at Riverside during a 3-week period ending October 16, 1936. This material yielded 110 adults of *Aneristus ceroplastae* How. and several specimens of *Marietta* sp. *A. ceroplastae* was propagated for only one generation, however, and none were colonized.[2]

The concurrent need for additional natural enemies for control of the black scale resulted in the shipment of large numbers of parasitized specimens of *Saissetia* spp. from Africa (Compere 1940)[3] to California during the winter and spring of 1937. These specimens yielded, among others, three species of parasitic Hymenoptera, *Coccophagus cowperi* Gir., *C. pulvinariae* Comp., and *Metaphycus helvolus*, each of which proved to be particularly adapted to the nigra scale. It is significant that the genus *Saissetia* is considered to be indigenous to Africa because of the presence there of numerous species of Hymenoptera known to be parasitic upon it (Compere 1940, Flanders 1953, van den Bosch *et al.* 1955). The probability that *S. nigra* is indigenous to Africa is based on two additional facts: (1) that it is rarely if ever an agricultural pest, although such plants as coffee, cotton, and guava are very favorable hosts; and (2) that on coffee in Kenya it is highly parasitized by one or more of six species of endoparasites even when very well attended by ants (Wilkinson 1929). In 1926, E. W. Rust, when in the Transvaal, collected nigra scale which yielded *Coccophagus isipingoensis* Comp. (Compere 1931).

The importation of natural enemies of nigra scale continued until May 27, 1940, when a shipment of parasitized nigra scale was received from Puerto Rico through the Bureau of Entomology and Plant Quarantine of the U. S. Department of Agriculture. This material consisted of nigra-scale-infested stems of *Hibiscus* sp. collected in Puerto Rico by K.A. Bartlett.[4] It is noteworthy that *Hibiscus* sp., the most favored host of nigra scale in the humid tropics (the Hawaiian Islands and Brazil) is in California immune to this scale (Smith 1944).

Of the natural enemies from Puerto Rico none was propagated or released in California. As a matter of record, these natural enemies are as follows: *Scymnus flavifrons* Welsh., *Scutellista cyanea*, *Lecaniobius cockerellii* Ashm., *Coccophagus scutellaris* (Dal.), and *Aneristus ceroplastae*.

Scutellista cyanea and *Coccophagus scutellaris* had existed in California for many years. *Lecaniobius cockerellii* reproduced in the laboratory for a generation or two but died out for lack of host material. *Scymnus flavifrons* had previously been repeatedly introduced into California without any evidence of its establishment (Flanders 1940). It is noteworthy that the life history of *Aneristus ceroplastae* is similar to that of *Coccophagus cowperi*.

In the field the nigra scale proved to be in general the most favorable host for the reproduction of the three species of African parasites. For their mass culture under laboratory conditions, however, the black scale, *Saissetia oleae*, because of its short life cycle is the most suitable host.

Coccophagus cowperi was the first species of natural enemy to be colonized on the nigra scale. This colonization took place on heavy infestations in Balboa Park, San Diego, in the fall of 1937. Within a few months the unsightly and apparently chronic infestations of this scale disappeared. In May 1938, the writer recovered *C. cowperi* from the release trees. In February 1941, Smith (1944) recovered it from nearby plants. The black scale, *Saissetia oleae*, does not appear to be a host of *C. cowperi* in the field. Under such conditions, however, this parasite reproduces readily on *S. hemisphaerica* (Targ.).

Coccophagus cowperi is a solitary parasite in scales which are about one-third grown. The male, which is a direct secondary ectoparasite, is produced only by the unmated female. The eggs deposited by such a female are fastened by means of a stalk to the outer surface of parasitic hymenopterous larvae or pupae enclosed in their cocoons within the host scale. The male and female larvae, during their first and second instars, differ greatly in structure since they are adapted to different types of environments. The female, when a prepupa, causes the dorsum of the host to turn prematurely black. At a temperature of about 80° F., the life cycle is 19 days.

Coccophagus pulvinariae was colonized during 1938 in black-scale-infested citrus orchards and on nigra scale infestations in dooryards. Its original establishment occurred on nigra-scale-infested *Pittosporum* plants in Santa Paula. In this locality it was again recovered in 1953 from the pepper tree, *Schinus molle*. Under field conditions in California the nigra scale is the only known host of *C. pulvinariae*. The life history of *C. pulvinariae* is similar to that of *C. cowperi*. When both species are reared together on *Saissetia oleae* in the same enclosure, *C. pulvinariae* ultimately replaces *C. cowperi*.

Coccophagus eritreaensis Comp., which biological studies show to be a color form of *C. pulvinariae*, was colonized in 1953 on nigra scale infesting California laurel (*Umbellularia californica*) on the University of California campus at Berkeley.

Metaphycus helvolus was colonized on nigra scale in Al-

[2] When in Sydney, Australia, in 1931, the writer found *Aneristus ceroplastae* to be a common parasite of lecanine scale insects. It highly parasitized *Coccus* spp. which occurred at low densities in the Botanical Gardens, but was relatively scarce in heavy infestations of *Saissetia* spp. on *Osteospermum moniliferum* grown along the seacoast. Harry S. Smith, in a letter dated April 29, 1931, in reference to this parasite stated: "This is a tropical parasite which I did not know occurred in Australia."

[3] In the fall of 1936, the University of California sent Harold Compere to Africa primarily in search of the parasite *Habrolepis rouxi* Comp., which at that time was the only species known to develop within the body of the California red scale, *Aonidiella aurantii* (Mask.).

[4] All the natural enemies of the nigra scale imported into California were received in quarantine by the writer, who then propagated for at least one generation the species to be released and made the initial colonizations in the field on infestations of nigra scale. Glenn L. Finney, Laboratory Technician, maintained the stocks of host material and mass-cultured certain natural enemies.

hambra, California, on May 11, 1938. A week later, material sent to Arleigh Kendall, Supervisor of the Los Angeles County Insectary, was released on native vegetation in Griffith Park, Los Angeles, on infested plants that were predominantly black instead of green as a result of the heavy covering of sooty-mold. Glenn Finney released 900 adults of *M. helvolus* in Griffith Park at about the same time. An additional colony of 350 specimens was released in Santa Monica on April 20, 1939.

The progress of *Metaphycus helvolus* in its destruction of the nigra scale in Griffith Park was followed by Glenn Finney and the writer until it appeared that none remained. The other host species continued to reproduce but at relatively low densities. An examination of the host plants in this area in the fall of 1957 showed this situation unchanged. Only in its immature instars is the nigra scale a host for *M. helvolus*. This parasite develops in hosts ranging from 0.6 to 1.6 mm. in length.

During the year ending in June 1939, when the nigra scale in the Los Angeles area was at the peak of abundance, large numbers of the young scale were being destroyed by predators, particularly by the lacewings, *Chrysopa californica* (Coq.) and *Hemerobius pacificus* Banks (Smith 1944).

THE FALL OF NIGRA SCALE.—By the end of the summer of 1939 the combined action of predators and parasites, plus the predatory action of adult female *Metaphycus helvolus* (Flanders 1942), had, in some locations, so reduced the scale population that it was difficult to find a live specimen (Smith 1944). On April 2, 1940, Ralph H. Smith reported to the writer (*in litt.*) that in the Los Angeles area *M. helvolus* was the only parasite found during the winter and up to that date. Four months later he found *M. helvolus* in considerable numbers on very small scales less than 1 mm. in length.

Thus in spite of heavy predator competition which destroyed parasitized and unparasitized scales alike, the parasite remained prevalent through 1940 and 1941. Consequently, as pointed out by Smith (1944), it constituted an important natural enemy of the scale.

The responsibility for the almost complete dying out of the nigra scale throughout most of the infested area from San Diego to Santa Barbara during the fall of 1939 and the early part of 1940 was attributed by Smith (1944) to the whole complex of natural enemies—not to any species in particular. However, it is significant that the nigra scale, prior to the establishment of *Metaphycus helvolus*, was generally on the increase in spite of all of its natural enemies. Only when *M. helvolus* became prevalent, beginning in 1940, did the nigra scale become permanently scarce.

Proof of the effectiveness of a natural enemy depends on the observed repetition of its controlling effect in either the same or different localities, and the dominance of that enemy at the lowest densities of the host.

During the years 1939 to 1941, inclusive, *Metaphycus helvolus* became increasingly effective in the southern coastal area of California, whereas in the northern coastal area where releases had been few and small in number, the populations of nigra scale were either static or on the increase. Smith reported September 12, 1940 (*in litt.*), that during the preceding 10 days he had found nigra scale abundant in one or two locations in Santa Barbara and in several places in San Luis Obispo. He found no parasites on nigra scale except *Scutellista cyanea*, which was abundant in places. The nigra scale appeared on the increase from Salinas northward.

Prior to September 1940, the only release of *Metaphycus helvolus* north of Santa Barbara took place in the summer of 1939. Small colonies were released on infestations of nigra scale on dooryard plants and on *Lecanium corni* Bouché in apricot orchards near the Bishop Homestead, a few miles south of the city of Santa Clara. This was at the time when the nigra scale was causing serious damage in nurseries in Santa Clara County. The lack of adequate colonizations apparently delayed the control of nigra scale north of Santa Barbara by *M. helvolus* for several years. Smith reported to the writer (*in litt.*) that a survey of the northern areas in September 1943, showed nigra scale was rather general from Salinas to San Francisco without any evidence of parasitization by *M. helvolus*. In Salinas he re-examined a large *Ilex* shrub which had been continuously and heavily infested with nigra scale for at least 3 years. It is noteworthy, according to Smith (1944), that from San Diego County to Monterey County nigra scale was most likely to be found on English ivy (*Hedera helix*), whereas from Monterey County to Sonoma County it was most likely to be found on English holly (*Ilex aquifolium*).

By the spring of 1941, the control of nigra scale along the south coast was so complete that the writer forecast (to Ralph H. Smith *in litt.*) that in the near future the nigra scale would be as well controlled by *Metaphycus helvolus* as is *Coccus hesperidum* L. by *M. luteolus* (Timb.). Observations by Steinweden (1945) and by county agricultural commissioners (*in litt.*) support this contention. The reports of several county agricultural commissioners as of August 1958, are paraphrased below:

San Diego County.—Nigra scale scarce despite climatic conditions which seem more favorable to this scale than other areas in the State.

Orange County.—During the late 1930's nigra scale heavily infested a great number of ornamentals and was increasing in importance until 1939. Since 1940 it has been of no importance.

Los Angeles County.—During the 10 years prior to 1941 the reported infestations of nigra scale numbered from 30 to 40 a year, but during the succeeding 17 years the reported infestations have averaged less than 1 a year.

Santa Barbara County.—In 1932 the nigra scale was a very common pest, particularly in nurseries. Now it is seldom found.

Santa Clara County.—Nursery inspections found only two or three infestations of nigra scale in 1957. These infestations were very light and occurred on ivy and holly, a striking contrast to the serious infestations of 20 years before.

Santa Cruz County.—No collections of nigra scale in plant nurseries during the past 2 years.

San Mateo County.—During the early 1940's nigra scale often heavily infested ornamentals throughout the county. Now it is rare indeed. Evidence of parasitization indicates that it is under biological control.

Sonoma County.—The nigra scale has not been reported

in the nurseries of this county during the past 10 years.

That *Saissetia nigra* had fallen to the status of *Coccus hesperidum* was confirmed in September 1958, by an inspector of the San Diego County Agricultural Commission, who discovered a rare, very light infestation on English ivy in Fern Canyon in the Zoological Gardens in Balboa Park. This infestation consisted of from 60 to 100 adult nigra scales and several hundred of their progeny. These progeny were highly parasitized by *Metaphycus helvolus*.

HABITS OF HOST AND PARASITE TENDING TOWARD ERADICATION OF THE HOST.—The final status of nigra scale in California may be much better than anticipated. At present this scale appears to have almost reached the point of extinction, at least in certain areas. There has been no published reference to this scale in California since Steinweden (1945) reported it as being effectively controlled by *Metaphycus helvolus* in the nurseries of the southern coastal area.

Theoretically, a parasite such as *Metaphycus helvolus*, which reproduces on several lecanine scales (*Saissetia oleae*, *S. hemisphaerica*, *Coccus hesperidum*, *C. pseudomagnoliarum* (Kuwana), and *Lecanium corni*), can eradicate a host such as *S. nigra* when it (the parasite) can perpetuate itself in large numbers in the same habitat on other species of scales.

According to Root (1924) "It is axiomatic that any parasite which inflicts serious injury on its host must necessarily be rarer than the host, else the host species and with it the parasite would be exterminated. This is true . . . particularly of parasitoid insect parasites."

Nicholson (1933) postulates this idea as follows: "The introduction of a new parasite species in general tends to reduce the steady density of the host species it attacks . . . and if it is a general parasite it may exterminate the host."

The reproductive habits of nigra scale as noted above render it particularly susceptible to attack by *Metaphycus helvolus*. Furthermore, unlike the other hosts of this parasite, its distribution in California appears limited to areas which are meteorologically favorable for the reproduction of the parasite. Consequently, it is possible that in some of these areas the nigra scale no longer exists.

In California, *Metaphycus helvolus* is not so effective on its other hosts, although the black scale in many localities is apparently held at noneconomic densities by *M. helvolus* (Smith 1942). In a number of localities, however, its control of the black scale is quite sporadic, and in some places completely lacking. *M. helvolus* failed to become established on black-scale-infested citrus at Canoga Park (Los Angeles County) in spite of repeated colonizations. It was observed prior to 1944 that its attack on black scale on olive in northern California was very erratic. It is evident that the parasitization of black scale by *M. helvolus*, unlike that of nigra scale, is "ecologically incomplete" (Flanders 1953). The same situation with regard to black scale may exist in Africa, supposedly the native habitat of *Saissetia* spp.

E. N. Rust, as foreign collector for the University of California, spent several years in Africa collecting the natural enemies of *Saissetia oleae*. He evaluated the importance of the various parasites in the control of this scale in Africa and rated *Metaphycus helvolus* as sixth, stating that "its work is very sporadic" (Smith & Compere 1926). He pointed out, however, that determination of the relative importance of the various black-scale parasites was difficult because of differences in the behavior of parasite populations in different localities and in the same locality in different seasons and years.

The reciprocal relation between the nigra scale and *Metaphycus helvolus* in California appears in itself to be optimum from the standpoint of the parasite as well as from that of the host, parasitization being physiologically and ecologically completely successful (Flanders 1953).

The rate of development of *Metaphycus helvolus* is such that it may have 10 generations to 1 of the nigra scale. An individual *M. helvolus* female has been observed to oviposit every day for 70 consecutive days, a total of 738 eggs, the maximum deposited on any one day being 18. The life of the adult female at out-of-door temperatures is unusually long. One female that emerged October 7, 1940, died on June 10, 1941. Such a female could be ovipositing when its progeny to the seventh generation are ovipositing. When hosts are so scarce that a female cannot oviposit for several weeks, she absorbs her ovarian eggs and ovigenesis ceases until hosts again are available (Flanders 1942). A parasite with such habits has great potentialities for maintaining its host at low densities, particularly a host such as the nigra scale.

Ordinarily, parasitization benefits a host species if such parasitization prevents the over population of the host and the consequent destruction of its food supply. However, if the host is part of a complex of host species with one or more species continually available to the parasite, then a particular species could be eliminated if its distribution is limited to localities most favorable for the reproduction of the parasite. Such an elimination of a host by a parasite may have occurred in certain localities in California with nigra scale and *Metaphycus helvolus*.

It is of interest to consider what effect *Metaphycus helvolus* might have had on the establishment of nigra scale in California if the parasite had been as prevalent prior to 1930 as it is today. In such a case the establishment of the scale would probably have been prevented by the parasite.

The prevention of insect establishment by the action of nonspecific entomophagous species already present may be a phenomenon of more general occurrence than is commonly recognized and in many instances a phenomenon of considerable economic importance. It is a common observation that exotic insects after their establishment acquire natural enemies. In North America the outstanding example is the acquisition of the indigenous *Macrocentrus ancylivorus* Roh. by the oriental fruit moth, *Grapholitha molesta* (Busck).

Entomophagous insects, along with their phytophagous hosts, have been carried by commerce from one part of the world to another. In this process many such species became established in new habitats, the nonspecific entomophagous forms in particular. Thus the nigra scale, upon its introduction into Puerto Rico, presumably was immediately subjected to attacks by the complex of nonspecific natural enemies, some of which were sent from there to California during 1940. In Puerto Rico these

natural enemies may prevent the nigra scale from attaining population densities detrimental to agriculture.

REFERENCES CITED

Compere, Harold. 1931. A revision of the species of *Coccophagus*, a genus of hymenopterous, coccid-inhabiting parasites. Proc. U. S. Natl. Museum. **78:** 1–132.

Compere, Harold. 1940. Parasites of the black scale, *Saissetia oleae*, in Africa. Hilgardia. **13:** 387–425.

Essig, E. O. 1931. The negro scale in California. Pan-Pacific Ent. **8:** 36.

Ferris, G. F. 1920. Scale insects of the Santa Cruz Peninsula. Stanford Univ. Publ. Univ. Ser. Biol. Sci. **1:** 1–57.

Flanders, Stanley E. 1940. A coccinellid introduced into California. Jour. Econ. Ent. **33:** 202–3.

Flanders, Stanley E. 1942. *Metaphycus helvolus*, an encyrtid parasite of the black scale. Jour. Econ. Ent. **35:** 690–8.

Flanders, Stanley E. 1953. Variations in susceptibility of citrus-infesting coccids to parasitization. Jour. Econ. Ent. **46:** 266–9.

Nicholson, A. J. 1933. The balance of animal population. Jour. Animal Ecol. **2:** 132–78.

Root, Francis Metcalf. 1924. Parasitism among insects. Sci. Monthly. **9:** 479–95.

Smith, Harry S. 1942. Biological control of black scale. California Citrograph. **27:** 266, 290–1.

Smith, Harry S., and Harold Compere. 1926. The establishment in California of *Coccophagus modestus* Silv. (Aphelinidae: Hymenoptera). Univ. California Publ. Ent. **4:** 51–61.

Smith, Ralph H. 1940. History of the nigra scale in California. California Dept. Agric. Quart. Bull. **29:** 102–5.

Smith, Ralph H. 1944. Bionomics and control of the nigra scale, *Saissetia nigra*. Hilgardia. **16:** 225–88.

Steinweden, J. B. 1945. Incidence of insect pests by counties for 1945. California Dept. Agric. Quart. Bull. **34:** 179–85.

van den Bosch, R., Blair, R. Bartlett, and Stanley E. Flanders. 1955. A search for natural enemies of lecanine scale insects in northern Africa for introduction into California. Jour. Econ. Ent. **48:** 53–5.

Wilkinson, H. 1929. Annual Report of the entomologist, 1928. Kenya Colony Dept. Agric. Ann. Rept. 1928: 172–86.

A Sequential Sampling Plan for Red-Pine Sawfly *Neodiprion nanulus* Schedl.[1,2]

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ABSTRACT

A recent outbreak of the red-pine sawfly, *Neodiprion nanulus* Schedl., has caused considerable damage to plantation red pine (*Pinus resinosa* Ait.) in northern New York State. A study of sampling methods for this pest led to the development of the sequential technique for use in control work. The experimental design, methods, and techniques used in the study are described, and a sequential sampling plan based on egg-infested needles found on 6-inch twig samples is presented. It was found, in testing the plan on field-collected data, that 45% in sampling time would have been saved had the plan been available for use at the time the field counts were made. Also, a test on the accuracy of the sequential plan showed that it failed only once in 25 trials to predict accurately the degree of defoliation to be expected at sampled locations. The confidence levels for the plan were set at 90%.

A recent outbreak of the red-pine sawfly, *Neodiprion nanulus* Schedl., has caused considerable damage to plantation red pine (*Pinus Resinosa* Ait.) in St. Lawrence County in northern New York. Light damage was first noted in 1952 in two widely separated locations. Since then the infestation has spread, and at present several thousand acres of red pine are being attacked.

The adult lays its eggs in the needle tissues during September and October. The eggs are laid singly in a line along the lengths of the needles. The eggs hatch the following May and the larvae feed gregariously on mature needles. They become fully grown in June at which time they spin cocoons in the duff at the base of the host tree and then rest until emergence occurs in the fall. A few may overwinter as pupae, emerging the following year.

In 1955 the New York State Museum and Science Service, in cooperation with the New York State Conservation Department and the United States Forest Service, initiated a study of methods of sampling for use in surveys of the red-pine sawfly in State plantations. This study led to the development of the sequential technique for use in control work.

Control of the larvae by airplane spraying with 1 gallon of 12% DDT in fuel oil (1 pound of DDT) per acre had proved satisfactory but the acreage requiring spraying remained questionable until observations on feeding larvae could be made in the field. Such delays often resulted in an inadequate control program, especially when large acreages were involved and there was only a brief period in which to make a quick survey.

The fact that the eggs of this pest are laid in the needles in the early fall and do not hatch until the following May offered a means of predicting damage before the larvae appeared and hence of allowing sufficient time to prepare a spray program, if one was found necessary.

METHODS AND PROCEDURES. In October 1955, a study area was set up in State Reforestation Area No. 6, Proposal C, Town of Brasher, St. Lawrence County. The plantation is primarily red pine, with some white pine and European larch. The proposal is 9 acres in size and was planted in 1934. It has experienced damage by red-pine sawfly since 1954.

In preparing the area for experimental study, 25 per-

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manent sampling locations were established, each with a group of five marked study trees. The trees were measured in 1956, when they averaged 30 feet in height and 8 inches in diameter. Their crowns averaged 23 feet in height and 11 feet in diameter. Some of the trees had been branch-pruned for crop trees to a height of 12 to 16 feet above the ground. The sampling locations or plots were evenly distributed over the area and were representative of its open borders as well as its interior.

Lateral twig samples were cut from the trees with a sectional pole pruner and examined for egg deposition. The pole-pruner method has been used successfully in measuring infestations of forest tent caterpillar, *Malacosoma disstria* Hbn. (Connola, *et al.* 1957) and spruce budworm, *Choristoneura fumiferana* (Clem.) (Morris 1954). Cut twigs of red pine showed that most of the red-pine sawfly eggs are laid in needles near tips of the branches. A standard measurement of 15 inches was established on a trial basis for the length of twig samples to be taken for study.

Five twig samples were taken at random from around the crowns of each of the five sample trees at each sampling location. The number of egg-infested needles on each sample twig was recorded and the needles were saved for egg-counting at the laboratory. Counts on over a thousand needles averaged 12 eggs per needle, with a standard deviation of ± 5. An average of 2 man-hours was required for cutting and examining the 25 twig samples per plot and counting the infested needles on them.

The twig sampling was followed by defoliation observations on the individual sample trees in the 25 sample plots the following June, after larval feeding was completed. A numerical system of recording defoliation was used in estimating foliage damage to the previous year's growth on individual trees. By this system, 1 represented 1% to 4% defoliation (trace), *2* was 5% to 25% defoliation (light), *3* was 26% to 50% defoliation (medium), *4* was 51% to 95% defoliation (heavy), and *5* was 96% to 100% defoliation (stripped). The above values were used to correlate egg deposit with foliage damage on the individual plots. It was necessary to confine defoliation readings to the previous year's needle growth because it was difficult to differentiate the older damage from current year damage.

SEQUENTIAL SAMPLING PLAN. Sequential analysis was applied to counts of infested needles on the twig samples from the 25 sampling locations according to the method described by Waters (1955). The counts closely fitted the negative binomial distribution. The statistic k for the pooled data was calculated by the regression method of Bliss & Owen (1958). The class limits were based on graphic study of the relationship between the number of infested needles found on 25 twigs per plot and plot defoliation. A quadrant sum test was applied to show this relationship and test its significance based on the data obtained.

Two classes are used in the plan. These classify an infestation as one in which the expected defoliation would be (1) zero to light or (2) moderate to heavy. The confidence levels were set at 90%. This would mean that there was 1 chance in 10 that a prediction by the plan would be in error.

Because of variations in infested needle counts and defoliation between the trees in individual plots as well as between plots, it was necessary to collect data such as described above for more than 1 year and submit them to sequential analysis. Thus it was possible to study weaknesses in the plan and to make improvements. The major improvement was a change from the 15-inch twig sample size to a 6-inch size. This resulted in a 50% saving in time required to count infested needles. It was found by count of almost 2,000 infested needles on 625 twig samples that 72% of the infested needles on a 15-inch twig were found on the first 6 inches of tip. Therefore it seemed logical that the 6-inch tip would be satisfactory as a relative measure of the insect population. Four plans were calculated between 1955 and 1958, two using the 15-inch twig size and two using the 6-inch size. Each of the plans was tested against its field data. Thus it was possible to see how accurate a particular plan was in its predictions had the plan been available when the field counts were made. Predictions were checked against actual defoliation at each plot and a tally was made of agreements and disagreements. The 1957–58 six-inch tip plan showed the greatest accuracy in its predictions. It was inaccurate in its prediction on one study plot out of the 25.

The plan is presented here (table 1), together with instructions on how to use it.

DISCUSSION. The main advantage to sequential sampling is the time saved in sampling without serious loss in accuracy. Tests with the plan presented against its field

Table 1.—Sequential sampling plan for red-pine sawfly egg populations on 6-inch red pine tips.

No. of Twig Samples	Cumulative Total Number of Egg-infested Needles		
	No. Expected to Produce Zero to Light Defoliation	Range Within Which the Amount of Defoliation Expected is Doubtful[a]	No. Expected to Produce Moderate to Heavy Defoliation
1	—	0–14	15 or more
2	—	0–16	17 or more
3	—	0–17	18 or more
4	—	0–19	20 or more
5	—	0–20	21 or more
6	—	0–21	22 or more
7	—	0–23	24 or more
8	—	0–24	25 or more
9	—	0–26	27 or more
10	0	1–27	28 or more
11	1 or less	2–28	29 or more
12	3 or less	4–30	31 or more
13	4 or less	5–31	32 or more
14	5 or less	6–33	34 or more
15	7 or less	8–34	35 or more
16	8 or less	9–35	36 or more
17	9 or less	10–37	38 or more
18	11 or less	12–38	39 or more
19	12 or less	13–40	41 or more
20	14 or less	15–41	42 or more
21	15 or less	16–42	43 or more
22	17 or less	18–44	45 or more
23	18 or less	19–45	46 or more
24	19 or less	20–47	48 or more
25	21 or less	22–48	49 or more

[a] Continue sampling if count falls in this column.

Note—Confidence levels specified for the plan are at the 90% level. The chances are 1 in 10 that predicted defoliation will be in error.

data showed that had we had the plan at the time of field sampling, we would have saved 45% sampling time by making decisions by the plan, instead of by the full complement of 25 samples per plot, as would have been the case using the conventional method of sampling.

There were 4 instances out of 25 when no decision was made by the plan. In other words, the counts fell in the doubtful (center) column and remained there throughout the 25-sample count. In such cases decisions were made on the basis of whether or not the count was closer to 21 (zero to light defoliation) or 49 (moderate to heavy defoliation). Of the four decisions made on this basis, only one was in error.

The desired accuracy of any plan is specified before the plan is calculated and is inserted into the calculations. However, the plan is only a tool and must be used properly to give satisfactory results. For instance, since most of the red-pine sawfly eggs are laid in the upper half of the crown, sampling from the upper crown or lower crown solely would give erroneous results. The 1958 defoliation data showed that defoliation averaged 22% in the upper halves of the crowns of study trees as compared with 5% in the lower halves. In our studies, the defoliation ratings in the upper and lower halves were averaged since the twig samples were taken at random around the crowns, but at the same time were representative of both upper and lower halves. The sampling of both upper and lower twigs apparently is the main reason for the variations in counts between samples within trees.

The egg deposition represents only a potential insect infestation. It is possible that eggs may not hatch, or larvae, for one reason or another, may not survive, in which cases predictions by the plan may not materialize. Such occurrences are in the realm of chance and must be expected. Nevertheless, the plan is useful in providing information which can be used for various purposes. In New York, the plan presented will be used during the fall and winter for determining the intensity and extent of red-pine sawfly infestations and the acreage to be sprayed the following spring where heavy damage is expected.

Instructions to be followed in the sequential sampling of red-pine sawfly eggs. (Refer to table 1.) The statistics involved in this plan are based on data collected in St. Lawrence County in 1957 and 1958. The sampling unit is a 6-inch lateral red pine tip. The sample is examined and the number of egg-infested needles is recorded.

The procedure in using the sequential plan for sampling is as follows:

1. Select a location for sampling red pine where there are at least five red pines in close proximity. If the red pines are numerous, select at random five trees which are next to each other.
2. The sampling of the trees should begin by taking the first twig sample from tree No. 1, the second from tree No. 2, the third from tree No. 3 and so on until five trees are sampled. Then return to tree No. 1 for the sixth sample, tree No. 2 for the seventh sample, and so on. The procedure then would be to sample each of the five trees at five sample intervals, always returning to the first tree and sampling through the fifth tree. This procedure is important as long as the plan calls for continued sampling.
   In using the plan it will be noted that if no infested needles are found, 10 samples must be examined before the site can be classified as one where no defoliation is to be expected. Where light defoliation is to be expected, the accumulated total number of infested needles must fall within the number indicated in that column. For example, if you examined 17 samples and found 9 or less infested needles, you would stop sampling and classify the site as one in which light defoliation is to be expected. If the count was 16 infested needles, you would continue sampling until the accumulated count fell in either the light defoliation column or the moderate to heavy column. Sampling may then cease at that site.
3. The distribution of sampling sites (stations) should be such that the sites are evenly distributed over an area and are representative of it. Thus sampling should be done in the interior of a plantation as well as along the edges. At least one sampling should be made in plantations 1 acre or less in size. In larger plantings 1 sampling for every 4 or 5 acres should suffice. If the acreage is very great, the sampling can be spread out farther, depending on time and manpower available for the work.
4. Twig sampling should be distributed evenly from the crowns of sample trees.

REFERENCES CITED

Bliss, C. I., and A. R. G. Owen. 1958. Negative binomial distributions with a common *k*. Biometrika **45**: 37–58.

Connola, D. P., W. E. Waters, and W. E. Smith. 1957. The development and application of a sequential sampling plan for forest tent caterpillar in New York. Bulletin No. 366, New York State Mus. and Sci. Serv., Albany, N. Y., pp. 1–22.

Morris, R. F. 1954. A sequential sampling technique for spruce budworm egg surveys. Canadian Jour. Zool. **32**: 302–13.

Waters, W. E. 1955. Sequential sampling in forest insect surveys. Forest Sci. 1(1): 68–79.

Diapause in *Anthonomus grandis* Boh.[1]

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Abstract

Part of the boll weevil, *Anthonomus grandis* Boh., population enters diapause during late summer and early fall in Louisiana. Winter is passed in this condition. Diapause in this insect is characterized by cessation of gametogenesis and atrophy of gonads, increase in fat content, decrease in water content, and decrease in respiratory rate.

Diapausing boll weevils were found in ground trash each month of the year except June and July. It is almost certain that they were also present there during these 2 months but no collections of ground trash were made at this time. Diapause occurred in some individuals as early as July 30 and movement to winter quarters began prior to August 16 during 1957.

Fat and water content remained relatively stable in diapausing weevils during late fall, winter and early spring. There was a sharp decrease in fat and a corresponding increase in water content soon after they moved back to cotton in the spring.

Males were able to resume spermatogenesis prior to leaving their winter quarters but oogenesis did not begin until the females had fed on seedling cotton.

The biology of the boll weevil, *Anthonomus grandis* Boh., has been studied intensively during that portion of the year when it is active. Comparatively little attention has been given to the insect during the period of inactivity during winter. It has been commonly accepted that the boll weevil hibernates as an adult during winter when food is not available. Favorite hibernatin sites in Louisiana have been shown to be the ground trash of forested areas and Spanish moss, *Tillandsia usneoides* L.

Observations made during the summer of 1956 led to the belief that the insect might undergo diapause instead of simply becoming inactive as a response to low temperatures encountered during the fall and winter months. Results of a study to investigate this possibility form the subject of this report.

A definition to fit all examples of delayed development which occur between the quiescence and "intense" diapause has not been proposed. It appears unlikely that a completely satisfactory definition can be formulated because there are so many borderline cases. One of the more satisfactory definitions of diapause is that of Simmonds (1948) who considers that diapause is a state " . . . in which a reduction of growth processes or maturation occurs which is not necessarily caused by immediate environmental influences, does not depend for its continuation on unsuitable conditions, and is not easily or quickly altered by change to a more favorable environment. However, once the state of diapause comes to an end normal growth and development are resumed." The physiological, ecological and phenological aspects of diapause have been discussed in general reviews by Andrewartha (1952) Bonnemaison (1944) and Lees (1955).

Diapause has been reported to occur in the adults of several species of Curculionidae. Among these are the apple-blossom weevil (*Anthonomus pomorum* L.) (Miles 1923), the vegetable weevil (*Listroderes costirostris obliquus* (Klug) (Dickson 1949), and the sweetclover weevil (*Sitona cylindricollis* Fahr.) (Davey 1956).

Lees (1955) listed failure of reproductive organs to enlarge, especially the ovaries, and hypertrophy of the fat body as morphological criteria of diapause in adult insects. Morphological differences between diapausing and active adults have been described in *Anthonomus pomorum* L. (Miles 1923), Colorado potato beetle, *Leptinotarsa decimlineata* Say (de Wilde 1953, 1954) and *Psylla pyri* L. (Bonnemaison & Missonier 1955).

The term hibernation as applied to the overwintering boll weevil was defined by Hinds & Yothers (1909) as "the entrance of the weevils upon a period of comparative if not complete inactivity." This could be construed as a loose definition of diapause. The importance of hibernation in the boll weevil has long been recognized (Hunter & Hinds 1904, Sanderson 1907, Hinds & Yothers 1909). Most of the work on hibernation has been done to determine preferred overwintering sites and the effect of environmental factors on survival. The standard procedure for studying winter survival has been to hold weevils collected in the fall in screen cages until the following spring (Hinds & Yothers 1909). In such experiments mortality has been found to be highest in the month immediately after introduction of weevils into the cages. For example, Sanderson (1907) reported that 51% of the mortality occurred during December, 9.6% during January and 2.8% during the remainder of the winter in weevils collected in the fall and held in screen cages.

Early fall destruction of cotton plants was first recommended as a control measure by Hunter & Hinds (1904). This practice has continued to be recommended because of the general belief that low temperatures during the fall force the boll weevil into hibernation (Hunter & Hinds 1904, Sanderson 1907). It has been generally observed that more weevils survive when placed in hibernation cages late in the season than early (Newell & Dougherty 1909). This has supported the theory that a long feeding period in the fall allows the boll weevil to accumulate sufficient food reserves to survive the hibernation period.

Methods of Procedure.—Weevils collected during the growing season were either hand picked or "shagged" by beating the plants over a large net. During the winter when the cotton plants were dead, weevils were collected from their hibernation sites in the ground trash of wooded areas adjoining cotton fields. The insects were separated from soil and larger bits of debris by shaking them mechanically through a series of screens and finally

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[2] Formerly Louisiana Agricultural Experiment Station, now Texas Agricultural Experiment Station.

[3] Louisiana Agricultural Experiment Station.

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placing the residue on a heated table which caused the weevils to move out of the trash within 5 to 10 minutes. Collections were made from ground trash at approximately monthly intervals from October 1956 to September 1957, except during June and July 1957. Periodic collections were made from one field each at Baton Rouge, Norwood, St. Joseph, and Tallulah, Louisiana, during 1957.

Weevils were dissected under Ringer's solution in wax-bottomed dissecting pans at 10× magnification. Elytra and hind wings were removed prior to dissection.

The following characters were studied: 1) Age: if the cuticle had hardened, the insect was considered old; if not, young, probably less than 3 days old. 2) Size was estimated visually and the insects classified as large, medium, or small. Those classed as large weighed more than 12 mg.; medium, 8 to 12 mg.; and small, less than 8 mg. 3) Fat was estimated visually by observation of the fat body through the abdominal terga. Individuals whose tracheae and internal organs were obscured by the fat body were classed as fat, intermediate if partially obscured, and lean if clearly visible. Fat was estimated chemically as ether soluble extractives. 4) Color of testes was classified visually as yellow, light yellow, or white. 5) A measure of the stage of spermatogenesis in the male was obtained by crushing a testis placed in Ringer's solution on a slide with pressure exerted on a cover slip and counting the number of sperm bundles in one field at 100× magnification. The number was considered to be many if 10 or more sperm bundles were present, few if less than 10, or none. The presence or absence of sperm in the testes and seminal vesicles was determined at the same time. 6) Size of testis was determined by measuring the least diameter of one lobe with an ocular micrometer. 7) Number of eggs in the ovaries was determined. All eggs in the lateral or median oviducts were classified as being mature; those in the vitellarium immature. 8) Length of ovaries from the tip of the ovariole to the point of evagination of the vaginal pouch was measured with an ocular micrometer. 9) Whether females had mated was determined by tearing the spermatheca and vaginal pouch apart in a drop of Ringer's solution and examining this preparation at 100× magnification for the presence of sperm. 10) Moisure content was determined by difference between live weight and dry weight. 11) Oxygen uptake was determined by the method described by Umbreit *et al.* (1949).

RESULTS AND DISCUSSION.—Boll weevils collected from the field during the fall of 1956 could be separated into two groups based on amount of fat accumulated. Some individuals had such marked hypertrophy of the fat body that neither the digestive tract nor the tracheae could be seen through the dorsum after removal of the elytra and hind wings. Others had accumulated little fat, and in these, parts of the digestive tract and the tracheae were clearly visible.

Differences in reproductive status of the two groups were revealed to be equally striking when they were dissected. The lean weevils were reproductively active exhibiting all stages of gametogenesis. Reproductive activity had ceased in fat individuals and the gonads showed marked regression.

Further study of the reproductive organs showed additional differences between fat and lean individuals. Testes of lean, reproducing males were white, opaque at the center and translucent around the edges. They were distinctly lobed and appeared fluffy. Their diameter ranged from 0.7 to 1.3 mm. All stages of spermatogenesis were present. The seminal vesicles were usually distended with sperm and chalky white in color. Accessory glands were enlarged.

Testes of fat, nonreproducing males were yellow, indistinctly lobed, filled with fat globules, dense in appearance, and ranged from 0.4 to 0.6 mm. in diameter. Al-

Table 1.—A comparison of reproductive activity, size, fat, diameter of testes, and length of ovaries of field- and trash-collected boll weevils. Louisiana 1956 and 1957.

Collected from	No. Weevils	Size			Fat			Sperm Bundles			Average Diameter Testes (mm.)
		Large	Medium	Small	Fat	Intermediate	Lean	Many	Few	None	
Males from Baton Rouge and Tallulah, 1956											
Field, July 31	22	7	15	—	—	—	22	22	—	—	0.99
Trash, Fall	25	10	12	3	22	3	—	—	—	25	.55
Males from Norwood, 1957											
Field, Nondiapausing	70	32	33	5	4	19	47	46	24	—	0.85
Field, Diapausing	25	17	7	1	13	10	2	—	—	25	.62
Trash, Diapausing	8	6	2	—	5	3	—	—	—	8	.61
Females from Baton Rouge and Tallulah, 1956											
								No. with Eggs			Average Length
								Mature		Immature	Ovaries (mm.)
Field, July 31	20	8	12	—	—	—	20	15		20	3.39
Trash, Fall	15	9	6	—	10	5	—	—		—	1.62
Females from Norwood, 1957											
Field, Nondiapausing	67	29	33	5	1	24	42	10		19	2.11
Field, Diapausing	17	14	2	1	17	—	—	—		—	1.61
Trash, Diapausing	7	6	1	—	2	5	—	—		—	1.54

FIG. 1.—Ovaries of diapausing (right) and reproducing female. (Photographed by J. H. Roberts.)

though some sperm was always present, active spermatogenesis had ceased as indicated by the absence of spermatocytes and the presence of few or no sperm bundles. Seminal vesicles were usually slender and lacked the chalky, white color characteristic in reproducing males. Accessory glands were comparatively short and slender.

Ovaries of lean, reproducing weevils ranged from 2.0 to 6.0 mm. or more in length when measured from the tip of the ovarioles to the point of evagination of the vaginal pouch. All stages of oogenesis were evident. In contrast, ovaries of fat, nonreproducing females ranged from 1.2 to 2.0 mm. in length and showed no evidence of oogenesis.

Detailed measurements showing comparisons of lean, reproducing weevils with fat, nonreproducing weevils are presented in table 1. Typical differences are illustrated in figures 1, 2, and 3.

Boll weevils collected from their hibernation quarters during the period of October 26 to December 4, 1956, were nonreproducing and predominantly fat. None of the females showed any evidence of oogenesis. Testes of most of the males showed the absence of spermatogenesis although some had a few sperm bundles present. All of the testes were yellow. Some of the insects collected during this period were quite lean but such individuals showed no evidence of gametogenesis. Characteristics of these weevils are presented in table 2.

Lees (1955) considered increased fat content and cessa-

FIG. 2.—Testes of diapausing (right) and reproducing male. (Photographed by J. H. Roberts.)

FIG. 3.—Testes preparation from a reproductively active male showing sperm bundles. (Photographed by J. H. Roberts.)

tion of reproductive activity the principal criteria of diapause in adult insects. Many of the weevils collected from the field during October and November 1956 exhibited these characteristics and were considered in diapause, table 2. All those taken from hibernation quarters in November and December 1956 were considered to be in diapause. They exhibited many of the same characteristics as those described for diapausing adults of *Anthonomus pomorum* (Miles 1923), *Leptinotarsa decimlineata* (de Wilde 1953, 1954) and *Sitona cylindricollis* (Davey 1956).

Further collections of diapausing weevils from ground trash made at approximately monthly intervals from October 1956 through May 1957 showed little change until late February when testes of some of the males had changed color from yellow to white, table 3. Resumption of spermatogenesis began during March when primary spermatocytes were observed in a few individuals. By April, spermatogenesis had proceeded to the extent that a few sperm bundles were observed in some of the testes. Most of the males collected from hibernation quarters during May had many fully developed sperm bundles.

That none of these males had fed was established by the absence of food in the gut when it was dissected and examined. This fact indicates that males are able to initiate spermatogenesis before leaving their hibernation quarters in the spring.

No evidence of oogenesis was found in any of the females collected from their hibernation quarters, table 3. Evidently the stimulus required for reinitiation of spermatogenesis in boll weevils which have diapaused differs from that required for oogenesis. This is not surprising since there is also a difference in the nondiapausing animals. Spermatogenesis begins during the pupal stage. Mature sperm bundles are present as soon as the male transforms to an adult and the seminal vesicles contain some sperm at this time. Oogenesis does not begin in the female for the first few days of adult life.

Approximately half the females collected from hibernation quarters during the period November 1956 to May 1957 had mated as indicated by the presence of sperm in the spermatheca and vaginal pouch, table 5. Motility of sperm decreased during the winter and by April the sperm in most of the females had degenerated and apparently was no longer viable. Examination of several samples of weevils just prior to formation of squares on the cotton indicated that approximately 100% of the females had mated during the current spring.

Boll weevils in diapause contained less water than reproductively active ones. Water content was 47 to 48% in weevils collected from ground trash during December, January, and February. It increased to 55.4% during April, table 2. Water content was 60 to 65% in populations in which all individuals were reproductively active. Decreased water content in diapausing insects has been reported for many species and seems to be a general phenomenon.

Respiratory rates of reproducing weevils were approximately three times greater than for diapausing ones. The oxygen consumption for trash collected weevils in which 100% were in diapause was 0.67 μl./mg./hr. For reproducing weevils the oxygen consumption was 1.85 μl./mg./hr. A field collection of weevils in which 50% were diapausing was run simultaneously and the oxygen

Table 2.—Boll weevils collected in the field during the fall of 1956 and in ground trash during fall and winter of 1956–57, in Louisiana.

Date Collected	Locality	No. Weevils	Sex Ratio M.:F.	Per Cent		Males		Females	
				Water	Fat	Active	Diapause	Active	Diapause
Oct. 15	St. Joseph	100	—	52.8	16.4	—	—	79	21
26	Baton Rouge	43	—	57.3	20.7	—	—	27	16
26	Tallulah	55	—	55.5	18.7	19	4	19	13
26	Tallulah[a]	11	5:6	—	—	0	6	0	5
30	Baton Rouge	28	—	56.5	18.0	9	3	8	8
Nov. 2	Baton Rouge	32	—	56.7	20.0	7	11	6	8
3	St. Joseph	46	—	—	—	18	2	17	9
9	Baton Rouge	32	—	—	—	16	3	9	4
10	Baton Rouge	40	—	—	—	12	7	11	10
15	Tallulah	62	38:24	—	—	24	14	18	6
16	Baton Rouge	32	—	—	—	12	1	16	3
Nov. 19–Dec. 4	Tallulah[a]	135	74:61	47.3	31.9	0	74	0	61
Jan. 8–10	Tallulah[a]	131	94:37	48.4	29.2	0	33	0	16
Feb. 26–27	Tallulah[a]	74	44:30	47.0	24.0	0	17	0	14
Mar. 5–29	Tallulah[a]	112	59:53	—	—	0	38	0	41
April 11	Tallulah[a]	75	48:27	55.4	25.7	1	14	0	15
May 24	Tallulah[a]	10	9:1	—	—	8	1	0	1

[a] Boll weevils collected in ground trash.

Table 3.—Male and female boll weevils collected in ground trash in 1956–57 classified visually for fat and reproductive activity of males and motility of sperm in mated females.

Date Collected	No. Weevils	Fat	Intermediate	Lean	Sperm Bundles: Many	Few	None
Males							
1956							
Oct. 26	5	4	1	—	—	—	5
Nov. 19–Dec. 4	74	45	22	7	1	14	59
1957							
Jan. 8–10	33	18	9	6	—	—	23
Feb. 26–27	17	7	2	8	—	—	17
Mar. 5–29	38	22	12	4	—	—	38
April 11	15	12	2	1	—	1	14
May 24	9	4	5	—	6	2	1
Aug. 16	8	5	3	—	—	—	8
Sept. 26	17	17	—	—	—	—	17
Totals	216	134	56	26	7	17	182
Females					No. Mated: Motile	Non-Motile	Unmated
1956							
Oct. 26	6	6	—	—	2	—	4
Nov. 19, Dec. 4	61	42	14	5	19	9	33
1957							
Jan. 8–10	16	5	9	2	2	9	5
Feb. 26–27	14	1	4	9	—	4	10
Mar. 5–29	41	20	15	6	8[a]	12	21
April 11	15	15	—	—	—	8	7
May 24	1	—	1	—	—	1	—
Aug. 16	7	2	5	—	7	—	—
Sept. 26	8	8	—	—	2	—	6
Totals	169	99	48	22	40	43	86 (51%)

[a] Very slight activity.

consumption was 1.09 μl./mg./hr.

No attempts were made to collect diapausing weevils from ground trash during June and July 1957. However, on August 16 collections were made in the vicinities of Norwood and Tallulah, Louisiana. Eight males and eight females were recovered, all of which were in diapause. Seventeen males and eight females were collected from ground trash near Tallulah, Louisiana, September 26. All these individuals were in diapause.

The number of weevils in their overwintering quarters at this time of year was quite surprising in view of many published reports stating that the insects are forced from the fields into winter quarters by low temperatures of the fall months (Hunter & Hinds 1904, Sanderson 1905, 1907, Hinds & Yothers 1909, Hunter & Pierce 1912). Fenton & Dunnam (1929) reported finding two boll weevils in Spanish moss collected September 1 to 3, 1925. This was prior to the occurrence of cool weather and the first killing frost, which occurred November 18.

It appears certain that boll weevils in diapause are present in woods throughout the year since weevils have been taken from winter quarters each month of the year (except June and July, when no collections were made) and since it is known that overwintered weevils frequently do not enter cotton fields in Louisiana before June and early July. Whether they are capable of remaining in diapause during an entire growing season and are able to resume activity the following growing season is not known.

Apparently the factors responsible for terminating diapause are not necessarily the same as those responsible for the insects leaving their winter quarters. Males collected from ground trash during April and May exhibited all stages of spermatogenesis. A few taken during the spring after they appeared on seedling cotton showed no evidence that spermatogenesis had begun. These individuals were still quite fat. Fat content of weevils taken from seedling cottong showed a progressive decline as the season advanced, figure 4. As the percentage of fat decreased water content increased.

Overwintered weevils were able to resume reproductive activity on food supplied by cotyledonary leaves and terminal buds of seedling plants prior to development of squares. Females matured eggs and males matured sperm on this diet. Vanderzant (1958) found that newly emerged weevils began ovipositing within 5 days when fed on cotton cotyledons.

Norwood Field.—This field chosen for a study of the seasonal occurrence of diapause was planted early. Low

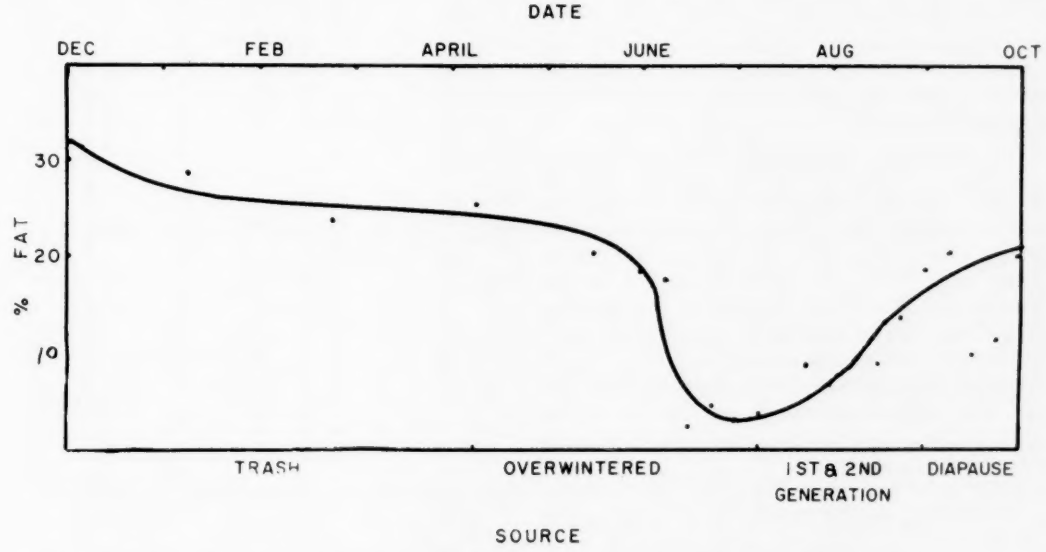

FIG. 4.—Seasonal variation in fat content of field populations of boll weevils in 1956–57.

level of fertility and droughty nature of the soil contributed to early fruiting. Weevil infestation remained at a low level until mid-July. Approximately 75% of the squares were punctured by July 20. Seven of 49 weevils collected July 30 were in diapause and 15 others were classified as being in an intermediate stage. By this time nearly all the squares were punctured and weevils were feeding heavily on bolls. Few, if any, weevils reared in bolls had emerged at this time. Plants had ceased growing and fruiting. Much fruit had shed, terminal buds had aborted, and a few of the older, undamaged bolls had begun to open.

The highest percentage of diapausing weevils collected from this field was found August 12. When duff from woods surrounding the field was sampled August 16, 16 specimens were found, all of which were in diapause. This showed that a heavy population of diapausing weevils had already entered hibernation quarters.

Only small squares in the terminals, foliage, and bolls were available as food for the heavy population of weevils remaining in this field during late August and early September. There was a flush of new growth during September, which produced another crop of squares. The percentage of diapausing weevils fluctuated with migration and with the fruiting activity of the plants during the remainder of the season.

Baton Rouge Field.—This field included three separate plantings at intervals of about 3 weeks, with the first being made in early April. Fertility level was high and fruiting was delayed. The first generation of weevils emerged during early July. No insecticide was applied, but the level of infestation was kept low by the constant removal of infested squares for use in other work. The population did not develop to the point that uninfested squares were scarce until late August. The first weevils in diapause were found August 29. At this time the infestation of squares was nearly 100%. Diapausing weevils were found in all the cotton at this time but more were present in the earliest planting. The highest percentage of diapausing weevils was found in this planting September 1 when comparatively few squares were present and heavy feeding on the bolls was evident.

Much movement from one planting to another was evident in this field. The percentage of weevils in diapause decreased after early September until October 17 and then showed an increase.

Tallulah Field.—Cotton was planted early at this location on Mississippi delta soil of high fertility. Weevils were not controlled effectively and a heavy, continuing infestation developed. The first generation began emergence in late June. Infestation throughout the season ranged from 75 to 100%. The first diapausing weevils were collected July 30 when the infestation was approximately 100% and heavy feeding on bolls was evident. The pouplation was drastically reduced by an application of methyl parathion dust immediately after this collection was made. Few diapausing weevils were found during August, but the percentage in diapause increased steadily through September and October.

No weevils were found in duff collected from the vicinity of this field August 16. Twenty-six specimens, all of which were in diapause, were collected when samples were taken near the same field September 26.

St. Joseph Field.—Late planting, high fertility, and excessive rainfall delayed fruiting in the field chosen for study at St. Joseph. No insecticide applications were made but this 5-acre field was surrounded by cotton under intensive insecticide treatment. Populations sufficient to allow collection of adults did not develop until mid-August. The infestation was still low during the first

Table 4.—Boll weevils collected in four fields in Louisiana during 1957 showing sex ratio, fat content and number diapausing determined by dissection.

Collected	No. Weevils	Sex Ratio (M:F)	Per Cent Fat	Males		Females	
				Active	Diapause	Active	Diapause
			Baton Rouge				
April 27	9	7:2	—	7	—	2	—
July 10	77[a]	37:40	—	18	—	22	—
11	91	44:47	7.3	22	—	20	—
24	97	61:36	9.2	20	—	20	—
31	103	55:48	6.9	38	—	28	—
Aug. 6	54	29:25	—	29	—	25	—
15	85	47:38	9.0	20	—	20	—
21[b]	77	53:24	14.1	38	—	9	—
21[c]	83	41:42	11.1	6	—	27	—
29[b]	81	49:32	19.3	20	5	20	2
29[c]	86	47:39	15.6	20	—	16	3
Sept. 1[b]	102	73:29	20.7	7	15	6	10
2[c]	43	17:26	—	16	1	20	—
5[c]	196	133:63	8.8	19	1	19	1
10	96	61:35	10.0	20	—	16	4
14	83	51:32	12.5	20	—	18	2
18	76	46:30	11.6	19	1	20	—
Oct. 4[c]	65	42:23	20.4	20	—	18	—
17	66	43:23	—	14	6	16	4
			Norwood				
May 3	18	11:7	—	11	—	7	—
6	43	32:11	—	29	3	11	—
20	352	242:110	13.8	27	—	20	—
June 3	75	55:20	14.6	18	—	11	—
July 30	92	58:34	22.8	25	5	17	2
Aug. 6	60	—	19.6	20	10	16	14
12	107	74:33	24.1	13	11	13	10
16[d]	16	8:8	—	0	8	0	7
20	93	60:33	22.8	15	6	13	5
26	87	54:33	14.9	17	10	12	10
Sept. 6	119	74:45	9.3	17	3	24	—
16	143	86:57	18.2	19	1	19	2
26[d]	—	—	—	—	—	—	—
Oct. 1	87	59:28	18.4	14	6	14	6
18	68	40:28	—	18	7	20	8
31	29	21:8	—	13	8	3	5
			St. Joseph				
May 20	41	30:11	—	25	—	6	—
27	38	25:13	18.7	25	—	13	—
June 3	95	69:26	11.0	26	—	14	—
18[a]	27	14:13	—	14	—	13	—
Aug. 13	73	44:29	7.4	26	—	17	—
19	50	28:22	6.7	11	—	9	—
26	51	26:25	2.7	15	—	12	—
Sept. 3	83	42:41	6.8	27	—	26	—
12	79	49:30	11.5	20	—	20	—
24	57	34:23	15.0	10	5	12	—
30	99	59:40	20.8	15	5	15	5
Oct. 7	72	38:34	—	20	—	20	—
22	54	26:28	—	17	9	13	15
28	51	—	—	10	16	11	14

[a] First generation.
[b] Old cotton.
[c] Young cotton.
[d] Trash collected.

Table 4.—(Continued)

Collected	No. Weevils	Sex Ratio (M:F)	Per Cent Fat	Males		Females	
				Active	Diapause	Active	Diapause
			Tallulah				
June 10	88	52:36	8.0	19	—	19	—
25[a]	43	26:17	4.6	—	—	—	—
July 1	108	60:48	4.0	24	—	23	—
8	111	68:43	6.9	41	—	20	—
15	105	63:41	6.4	21	—	20	—
23	109	62:47	5.2	22	—	21	—
30	129	75:54	10.3	26	1	22	1
Aug. 5	60	—	10.1	30	—	30	—
13	85	45:40	11.3	21	—	18	1
16[d]	—	—	—	—	—	—	—
20	100	53:47	23.5	20	—	23	2
27	66	37:29	—	17	4	7	8
Sept. 4	124	77:47	23.7	16	4	20	2
23	79	46:33	28.3	15	5	14	6
26[d]	25	17:8	—	0	17	0	8
Oct. 7	73	42:31	27.5	14	6	11	9
28	55	34:21	—	12	9	12	9

half of September. The first diapausing weevils were collected September 24, almost 2 months later than at Tallulah 40 miles to the north and Norwood about 100 miles to the south. Infestation of squares was virtually complete but no feeding was evident on the relatively few young bolls present. Data showing seasonal occurrence of diapause in these four fields are summarized in table 4 and illustrated in figure 5.

Diapausing weevils were collected in the fields at Norwood and Tallulah July 30, but did not occur in the other two fields until a month later at Baton Rouge and almost 2 months later at St. Joseph. Baton Rouge is approximately 40 miles south of Norwood and St. Joseph is about the same distance south of Tallulah.

Data summarized in table 5 were obtained from miscellaneous collections made in various localities of Louisiana, Mississippi, Missouri, Texas, and Mexico. Six collections were made in the vicinity of Brownsville, Texas. The earliest of these was made May 10. Collections were obtained from Manuel and Valles near Tampico, Mexico, during the period January 30 to April 4. All these collections contained some specimens in diapause.

Thurberia Weevil.—One sample of the thurberia weevil *Anthonomus grandis thurberiae* Pierce was obtained from Arizona July 3. These weevils were still in their pupal cells in the bolls of *Gossypium thurberi* in which they were collected. Although they had probably been adults since the preceding fall and had not fed since that time, they contained 16.1% fat. A striking difference between these weevils and the boll weevil was the absence of sperm in the testes and seminal vesicles of the male. Some sperm was always present in both reproducing and diapausing male boll weevils even though no other evidence of spermatogenesis was discernible. Sperm could be found in both the testes and seminal vesicles of the boll weevil immediately after transformation to the adult.

Sex Ratio.—A consistent difference in the sex ratio of diapausing boll weevils of about 1.5 males to 1 female was found. The ratio was approximately 1:1 in reproducing

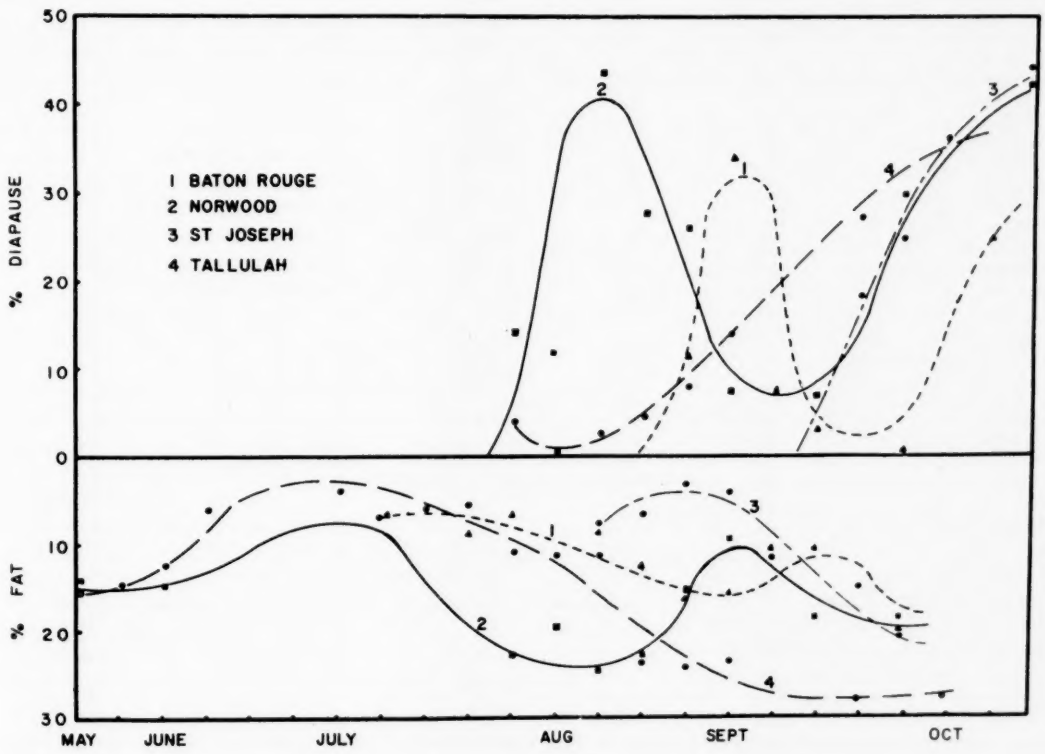

FIG. 5.—Seasonal incidence of diapause and fat content of boll weevils in four fields located at Baton Rouge, Norwood, Tallulah and St. Joseph, Louisiana, during 1957.

populations. Hunter & Hinds (1904) reported that the ratio was about equal except that more males than females were found among hibernating weevils. Hinds & Yothers (1909) found about 54% of overwintered weevils were males and Hunter & Pierce (1912) reported almost equal numbers of males and females during the fall before their entry into hibernation. Data summarizing sex ratios during different periods of seasonal development are given in table 6.

The significance of the difference in sex ratio of diapausing and reproductively active populations is not understood. Limited data from tanglefoot trap collections and from one isolated field which was infested by migrating weevils indicate that more females than males fly

Table 5.—Miscellaneous field collections of boll weevils during 1957 with sex ratio, fat content and number diapausing determined by dissection.

Date Collection and Locality	No. Weevils	Sex Ratio (M:F)	Per Cent Fat	Males		Females	
				Active	Diapause	Active	Diapause
May 10, Brownsville, Tex.	116	80:36	22.0	43	9	15	5
June 12, Brownsville, Tex.	301	187:114	9.8	35	5	15	1
June 25, Brownsville, Tex.	87	55:32	17.7	24	2	14	3
July 3, Brownsville, Tex.	300	181:119	22.0	22	2	15	6
Aug. 5,[b] Brownsville, Tex.	47	34:13	—	14	8	7	6
Aug. 5,[a] Brownsville, Tex.	50	28:22	—	27	1	20	2
Jan. 30, Matamoras, Mexico	74	—	16.7	56	1	17	—
Feb. 26, Matamoras, Mexico	97	55:42	15.7	33	2	21	—
April 4, Matamoras, Mexico	125	91:34	17.8	44	1	23	—
Feb. 5, Manuel, Mexico	169	99:70	16.3	26	5	30	—
Feb. 5, Valles, Mexico	184	94:90	19.6	32	1	30	—
March 30, Valles, Mexico	132	76:56	26.0	38	13	32	14
May 24,[c] Waverly, La.	144	95:49	15.7	23	—	20	—
June 3,[c] Waverly, La.	125	91:34	9.0	21	—	20	—
June 10,[c] Waverly, La.	119	89:30	8.0	20	—	15	—
June 17,[c] Waverly, La.	99	64:35	2.6	32	—	17	—
June 25,[c] Waverly, La.	119	90:29	4.8	24	—	23	—
July 1,[c] Waverly, La.	124	87:37	3.3	22	—	21	—
July 8,[c] Waverly, La.	92	67:25	3.9	20	—	19	—
May 24,[c] Tallulah, La.	143	94:49	16.0	19	—	21	—
May 29,[c] Tallulah, La.	150	98:52	14.4	31	—	13	—
June 3,[c] Tallulah, La.	108	67:41	12.4	20	—	21	—
June 6,[c] Tallulah, La.	201	129:72	—	20	—	20	—
June 10,[c] Tallulah, La.	141	88:53	6.0	24	—	12	—
Aug. 13, Tallulah, La.	62	32:30	8.6	17	—	15	—
Aug. 20, Tallulah, La.	82	51:31	9.3	25	2	14	2
Aug. 20, Tallulah, La.	114	60:54	10.9	14	—	13	—
Aug. 26, Tallulah, La.	64	18:46	10.9	9	1	20	3
Aug. 26, Tallulah, La.	141	66:75	14.4	20	3	16	5
Sept. 3, Tallulah, La.	105	57:48	13.9	22	3	21	1
May 30,[c] Dubach, La.	39	22:17	—	22	—	17	—
June 18,[c] Dubach, La.	105	58:47	3.6	30	—	29	—
June 23,[c] Dubach, La.	105	62:43	4.4	41	—	22	—
July 9, Dubach, La.	23	16:7	—	16	—	7	—
July 23, Dubach, La.	21	10:11	—	10	—	11	—
Aug. 13, Dubach, La.	12	3:9	—	3	—	9	—
Aug. 27, Dubach, La.	144	81:63	14.1	47	2	44	5
Sept. 23,[a] Dubach, La.	49	24:25	—	13	2	14	1
Sept. 23,[b] Dubach, La.	36	24:12	—	10	5	12	—
May 7,[c] Livonia, La.	85	48:37	11.8	19	1	20	—
May 9,[c] Bunkie, La.	35	22:13	—	22	—	13	—
May 14,[c] Lafayette, La.	57	38:19	14.1	38	—	19	—
June 6,[c] Leton, La.	89	58:31	—	26	—	14	—
Aug. 3, Bay City, Tex.	51	27:24	—	14	5	14	1
Aug. 4, Kingsville, Tex.	41	—	—	13	8	17	3
Aug. 4, Sinton, Tex.	45	22:23	—	21	—	21	1
Aug. 8, Hearne, Tex.	57	34:23	—	34	—	22	1
Aug. 8, College Station, Tex.	60	37:23	—	37	—	23	—
Sept. 6, Centerville, Miss.	80	56:24	7.3	22	—	18	—
Sept. 18, Erath, La.	99	61:38	10.4	17	3	19	1
Oct. 4, Sikeston, Mo.	102	57:45	18.0	18	2	12	8
Oct. 24, Sikeston, Mo.	40	—	—	7	13	6	14
July 3,[d] Arizona	82	50:32	16.1	0	9	0	9
Aug. 22, Baton Rouge, La.[a]	125	56:69	—	56	—	69	—

[a] Young cotton.
[b] Old cotton.
[c] Overwintered weevils.
[d] Thurberia weevil.

Table 6.—Sex ratio of adults collected at different periods of the seasonal development of the boll weevil population.

Description	Total	Males		Females	
		No.	Per Cent	No.	Per Cent
Trash collected	589	359	61	230	39
Field collected, overwintered	2629	1770	67	859	33
Field collected,[a] before diapause	2072	1146	55	926	45
Field collected, after diapause	5279	3155	60	2124	40
Reared from squares collected in the field	1100	569	52	531	48
Reared from bolls collected in the field	249	109	44	140	56
Tanglefoot traps before diapause	69	28	41	41	59
Tanglefoot traps after diapause	19	12	63	7	37

[a] Includes all weevils collected after first generation weevils were present in the field, therefore this record could include some overwintered individuals.

from one field to another prior to the onset of diapause. The reverse seems to be true afterward. Data in table 6 show that approximately equal numbers of the sexes emerge from infested bolls and squares collected in the field and brought into the laboratory. Low temperatures favored development of males in rearing experiments (Hunter & Pierce 1912).

It seems probable that the factors which induce diapause in the male are not the same as for the female, or that the level of stimulation is different. Half the females collected from their hibernation quarters during 1956–57 had not mated, which indicated that these individuals had not reached sexual maturity prior to the onset of diapause. Whether those which had mated, as indicated by the presence of sperm in the vaginal pouch and spermatheca, had reproduced prior to their entry into diapause is not known. Since many females collected in the field contained eggs which appeared to be in the process of being resorbed, it is possible that some may reproduce prior to entering diapause. Nevertheless, none of the females collected from their hibernation quarters showed any evidence of ever having initiated oogenesis.

Fat Content.—Seasonal variation in fat content of the boll weevil populations studied is summarized in tables 1, 4 and 5, and illustrated graphically in figures 4 and 5. These data show that fat reserves are depleted very slowly in the diapausing animals during the winter. Fat content of weevils collected from seedling cotton at Waverly, Louisiana, still amounted to about 20% through the first week of June. By the middle of June it had dropped to about 5%. Fat reserve of the overwintered population was at its lowest level after the females began oviposition. Fat content was comparatively low during the first generation but increased steadily thereafter. It amounted to about 20% by the time the first diapausing weevils were observed.

Other Variables.—The drastic physiological and morphological changes which occur when the boll weevil enters diapause provide an explanation for the seasonal variation in susceptibility to insecticides reported by Rainwater & Gaines (1951).

Only weevils in diapause were found to overwinter. This shows that the hibernation cage procedure which has been commonly used to determine winter survival may be quite misleading and is of doubtful value. Since both reproducing and diapausing weevils are present in the fields when collections with which to stock hibernation cages are made, survivial will be correlated with the percentage of the population in diapause. This may vary from field to field and even from one part of a field to another.

Presence of diapausing boll weevils in hibernation quarters throughout the year and the apparent continous emergence from these quarters during the growing season offers an explanation for the lack of success in many areas of both early season insecticide applications and fall stalk destruction programs for control of the boll weevil.

References Cited

Andrewartha, H. G. 1952. Diapause in relation to the ecology of insects. Biol. Rev. **27:** 50–107.

Bonnemaison, L. 1944. Arrets de Developpement et Diapauses. Ann. Epiphyt. et Phytogen. n.s. **10:** 19–56.

Bonnemaison, L., and Missionier, J. 1955. Recherches sur le determinisme des formes estivales ou hivernales et de la diapause chez le psylle du poirier (*Psylla pyri* L.) Ann. Epiphyt. **6:** 457–528.

Davey, K. G. 1956. The physiology of dormancy in the sweet clover weevil. Canadian Jour. Zool. **34**(2): 86–98.

deWilde, J. 1953. Diapause in Colorado potato beetle. Acta Physiol. et Pharmacol. Neerland **3:** 141.

deWilde, J. 1954. Aspects of diapause in adult insects with special regard to the Colorado beetle, *Leptinotarsa decimlineata* Say. Arch. Néerl. Zool. **10:** 375–85.

Dickson, R. C. 1949. Factors governing the induction of diapause in the oriental fruit moth. Ann. Ent. Soc. America **42**(4): 511–37.

Fenton, F. A., and E. W. Dunnam. 1929. Biology of the cotton boll weevil at Florence, South Carolina. U. S. Dept. Agric. Tech. Bull. **112.**

Hinds, W. E., and W. W. Yothers. 1909. Hibernation of the Mexican cotton boll weevil. U. S. Dept. Agric. Bur. Ent. Bull. **77.**

Hunter, W. D., and W. E. Hinds. 1904. The Mexican cotton boll weevil. U. S. Dept. Agric. Bur. Ent. Bull. **45.**

Hunter, W. D., and W. D. Pierce. 1912. Mexican cotton boll weevil, U. S. Senate Document No. 305. U. S. Dept. Agric. Bur. Ent. Bull. **114.**

Lees, A. D. 1955. The physiology of diapause in Arthropods. University Press, Cambridge.

Miles, H. W. 1923. Observations on the bionomics of the apple-blossom weevil, *Anthonomus pomorum* L. Ann. Appl. Biol. **10:** 348–69.

Newell, W., and M. S. Dougherty. 1909. The hibernation of the cotton boll weevil in central Louisiana. Louisiana Crop Pest Comm. Circ. **31:** 163–219.

Rainwater, C. F., and J. C. Gaines. 1951. Seasonal decline in the effectiveness of certain insecticides against the boll weevil. Jour. Econ. Ent. **44**(6): 974–6.

Sanderson, E. D. 1905. Some observations on the cotton boll weevil. Proc. 17th Ann. Meeting Assoc. Econ. Ent. U. S. Dept. Agric. Bur. Ent. Bull. **52:** 29–42.

Sanderson, D. E. 1907. Hibernation and development of the cotton boll weevil. U. S. Dept. Agric. Bur. Ent. Bull. **63,** pt. 1.

Simmonds, F. J. 1948. The influence of maternal physiology on the incidence of diapause. Trans. Roy. Soc. London (Series B), **233**(603): 385–414.

Umbreit, W. W., R. H. Burris and J. F. Stauffer. 1949. Manometric techniques and tissue metabolism. Burgess Publishing Co., Minneapolis, Minn.

Vanderzant, Erma S., and T. B. Davich. 1958. Laboratory rearing of the boll weevil. A satisfactory larval diet and oviposition studies. Jour. Econ. Ent. **51**(3): 288–91.

Damage to Corn in Louisiana Caused by Stored-Grain Insects[1]

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Abstract

A 3-year study of damage to corn by stored-grain insects showed that infestation of standing corn in Louisiana by the rice weevil (*Sitophilus oryza* (L.)) was extremely high. The average damage for the State at harvest was found to be approximately 10% kernel damage. This damage gradually increased during storage until in May it was 17% and in July the average damage was more than 30% kernel damage. Damage by other species was negligible except for the Angoumois grain moth (*Sitotroga cerealella* (Oliv.)). Field infestation by this species at harvest represented less than 2% of the total damage; however, by midsummer it was responsible for 16% of the total damage. The square-necked grain beetle (*Cathartus quadricollis* (G.-M.)) was found to be extremely abundant and present in nearly all ears. Other species such as the red flour beetle (*Tribolium castaneum* (Hbst.)), the saw-toothed grain beetle (*Oryzaephilus surinamensis* (L.)) and the flat grain beetle (*Laemophloeus minutus* (Olivier)) were found but not as abundantly as the square-necked grain beetle. Damage was found to vary by years as well as by areas.

A tight or sound husk covering was found to provide a considerable degree of protection to corn from infestation by the rice weevil and the Angoumois grain moth.

In Lousiana damage to standing corn in the field and progressive loss during storage caused by stored-grain insects is excessive.

Observations and control studies (Floyd *et al.* 1958) in the field both previous to and at harvest have shown that corn while in the dough stage is often infested by rice weevils (*Sitophilus oryza* (L.)) in Louisiana. This infestation is nearly always associated with great numbers of square-necked grain beetles (*Cathartus quadricollis* (G.-M.)). Powell (1958) showed that the rice weevil can develop in corn in the field at a much higher grain moisture content than heretofore considered possible.

Oviposition which occurred at a grain moisture of 65% resulted in larval development and emergence of normal adults. Eggs, if laid in kernels of higher grain moisture, did not develop.

Corn, which is not a cash crop in Louisiana, is usually left in the field until cotton is harvested. As a result, it is often November or later before corn is stored. Delayed harvest after early field infestation by rice weevils in immature corn, and damage to the husks by birds and rodents are prediposing factors which favor heavy infestation at the time of harvest. Recent studies in Louisiana (Floyd *et al.* 1958) have shown that damage to the shuck caused by birds and the corn earworm (*Heliothis zea* (Boddie)) facilitate initial entry of the rice weevil into ears of corn in the field in Louisiana.

Data presented herein result from a study initiated in the fall of 1955 to determine the damage to corn at harvest and after several months of storage. The general plan called for a division of the State into seven natural geographical areas. Samples of corn were obtained from the same farms in each area in the fall immediately after harvest, and in May and July of the following year.[2] Examination of the snapped ears was done as follows: Each ear was classified relative to its husk condition—good or poor. An ear with husks sufficiently open on the end or sides to render the kernels visible was classified as poor. Good husk condition was represented by an ear with husks closed sufficiently tight at the tips to obstruct vision of the kernels and which was void of damaged areas on the sides caused by birds, rodents, or earworms. After husk condition was classified the husks were removed. The ears were then grossly examined to determine if larval feeding channels of the rice weevil were present or had been present and for presence of emergence holes made by adults. Emergence holes made by the Angoumois grain moth (*Sitotroga cerealella* (Oliv.)) were also recorded. Presence of adult rice weevils on the ears was not used to determine ear infestation by this species since movement of the adult from ear to ear or from sample to sample before they were examined was quite possible. However, the presence of such species as the square-necked grain beetle, the saw-toothed grain beetle (*Oryzaephilus surinamensis* (L.)) and the flour beetles on each ear was considered a valid criterion of infestation since these species are not disturbed as easily as the rice weevil. The square-necked grain beetle was not counted separately from other miscellaneous beetles during the 1955–56 examinations; however, in later examinations this separation was made. The miscellaneous beetles group included the square-necked grain beetle, the red flour beetle (*Tribolium castaneum* (Herbst.)), and the rusty grain beetle (*Laemophloeus ferrugineus* (Steph.)). Other species such as the Indian-meal moth, (*Plodia interpunctella* (Hbn.)) the meal worms, and the cadelle (*Tenebroides mauritanicus* (L.)) were found only in grain that was in poor condition or stored for a long time.

After the husked ears were examined for infestation by the various species they were shelled. The percentage of kernels damanged by the rice weevil and the Angoumois grain moth was determined by examination of 1,000 kerkernels per sample in lots of 100. Each lot of 100 kernels was viewed under an illuminated magnifying lens. Rice weevil damage was determined by counting the kernels which showed larval feeding channels and/or emergence holes made by adults, and feeding damage caused by adults. Damage by the Angoumois grain moth was determinted by the presence of emergence holes made by emerging adults. At this time the grain moisture and the weight per bushel were determined.

No attempt was made to classify the different varieties of corn represented. Only storage type varieties or hybrids were used. "Hogging-off" types in Louisiana are usually destroyed by insects and rot by the time they reach maturity.

The seven geographical areas into which the State was

[1] Accepted for publication December 22, 1958.

[2] W. T. Spink, Louisiana Agricultural Experiment Station, assisted in collecting the samples for the summer, 1958, examination.

divided were as follows: (1) the Ouachita Valley located in northeast Lousiana; (2) the Red River Valley, beginning in the central part of the State and extending to the northwest corner; (3) the north Louisiana hill country between areas 1 and 2; (4) the Florida parishes (Southeast Louisiana exclusive of the Mississippi Delta south of New Orleans); (5) southwest Louisiana; (6) The Mississippi River Valley beginning at Morganza and extending north to the Arkansas line; and (7) the Mississippi River Valley beginning at Morganza and extending south to the Gulf of Mexico.

Plans for the survey required a minimum of 10 samples from as many farms, consisting of 20 ears each to be taken from each area for the fall or harvest examination. Subsequent examinations of like samples were made from as many of the same farms and same storage cribs as possible. It is a common practice among Louisiana farmers to purposely feed most of the corn to livestock before summer approaches in order to avoid excessive insect damage. For this reason it was considered likely that the number of samples taken in the spring and summer examinations would be fewer than those obtained in the fall.

Insect control in stored corn in Louisiana is generally not practiced. A few farmers use DDT or cotton-poison formulations scattered among the ears in storage to suppress weevil damage. Very rarely is corn fumigated. Treated cribs were not sampled.

The 20-ear sample was taken at random from the top of the pile to the floor of the bin. Ears in storage were nearly always aligned in such a position that they presented more or less of a wall. This manner of selection was ideal for obtaining a reasonably representative sample.

RESULTS.—The data given in tables 1, 2, and 3 show damage by species and/or groupings of species for each area for 1955–56, 1956–57, and 1957–58. Only two sampling dates are shown for the 1957–58 season. The effect of the husk condition as classified in this study on infestation of the ears by four species of stored-grain insects is shown in table 4. Data in table 5 show the effect of insect damage (largely rice weevil) on the weight per bushel disregarding the grain moisture-loss factor.

DISCUSSION.—The figures presented in tables 1, 2, and 3 show that the damage to corn in Louisiana at harvest was extremely high, and that this damage progresses in storage until midsummer when the grain quality is greatly reduced. Locally it is termed "pig-feed" at this time. The damage at harvest is caused almost exlusively by the rice weevil. Damage during storage was augmented by the Angoumois grain moth. In July, 16% of the total damage was attributed to this latter species.

It is of interest to note the area differences in damage caused by the rice weevil in Louisiana. The State was not uniformly infested. In the fall of 1955 area 1 was practically free from damage. In 1956 damage was greater but it still had the lowest infestation in the State. The 1957 examination showed an additional increase in initial damage nearer to the State average. Corn in areas 2 and 6 (the Red River and the upper Mississippi River valleys respectively) was less damaged than in the other areas, but was damaged more than corn in area 1. These differences

Table 1.—Infestation in corn by stored-grain insects. Louisiana, 1955–56.

	AREAS OF STATE							STATE AVERAGE
	1	2	3	4	5	6	7	
				Harvest, 1955				
Per cent ears infested by:								
Rice weevil	2	24	28	58	62	26	51	36
Angoumois grain moth	1−	3	11	1−	1−	4	1−	3
Misc. beetles[a]	12	39	30	63	74	43	61	46
Saw-toothed grain beetle	1−	7	6	9	9	9	11	7
Per cent kernel damage	1−	3	4	13	26	5	9	9
Per cent damage caused by:								
Rice weevil	98+	98+	98+	98+	98+	98+	98+	98+
Angoumois grain moth	2−	2−	2−	2−	2−	2−	2−	2−
				May, 1956				
Per cent ears infested by:								
Rice weevil	8	27	42	55	76	32	62	43
Angoumois grain moth	6	19	32	2	1−	22	12	13
Misc. beetles[a]	1	16	6	46	57	13	40	26
Saw-toothed grain beetle	0	2	2	0	0	1−	1	1
Per cent kernel damage	1	7	7	24	26	6	24	14
Per cent damage caused by:								
Rice weevil	98	100	98	100	100	98	100	99
Angoumois grain moth	2	—	2	—	—	2	—	1
				July, 1956				
Per cent ears infested by:								
Rice weevil	23	44	78	55	72	35	64	53
Angoumois grain moth	22	57	41	11	0	28	9	24
Misc. beetles[a]	1	26	37	44	7	9	22	21
Saw-toothed grain beetle	0	5	0	0	1	1	2	1
Per cent kernel damage	6	13	40	14	36	12	33	22
Per cent damage caused by:								
Rice weevil	75	75	75	90	100	75	95	84
Angoumois grain moth	25	25	25	10	—	25	5	16

[a] Includes the red flour beetle and the square-necked and flat grain beetles.

Table 2.—Infestation in corn by stored-grain insects. Louisiana, 1956–57.

	Areas of State							State Average
	1	2	3	4	5	6	7	
				Harvest, 1956				
Per cent ears infested by:								
Rice weevil	8	18	47	49	41	4	54	32
Angoumois grain moth	5	5	24	21	3	24	9	13
Misc. beetles[a]	1	4	5	4	8	4	5	4
Square-necked grain beetle	42	38	57	40	55	18	52	43
Saw-toothed grain beetle	15	30	20	10	16	22	11	18
Per cent kernel damage	1−	3	10	13	9	3	13	7
Per cent damage caused by:								
Rice weevil	99+	99+	99+	99+	99+	99+	99+	99+
Angoumois grain moth	1	1	1	1	1	1	1−	1−
				May, 1957				
Per cent ears infested by:								
Rice weevil	26	49	72	77	74	46	64	58
Angoumois grain moth	13	23	47	46	11	69	23	33
Misc. beetles[a]	13	22	25	44	21	29	46	29
Square-necked grain beetle	43	62	71	81	64	25	51	57
Saw-toothed grain beetle	33	39	45	53	31	66	52	46
Per cent kernel damage	3	12	24	33	29	15	30	21
Per cent damage caused by:								
Rice weevil	80	98	93	99+	99+	53	99+	89
Angoumois grain moth	20	2	7	1−	1−	47	1−	11
				July, 1957				
Per cent ears infested by:								
Rice weevil	67	62	85	75	96	68	66	74
Angoumois grain moth	48	20	21	3	1	92	3	27
Misc. beetles[a]	41	54	91	65	93	91	60	71
Square-necked grain beetle	34	17	7	7	18	4	1−	13
Saw-toothed grain beetle	13	19	30	27	25	42	21	25
Per cent kernel damage	26	27	59	41	51	49	32	41
Per cent damage caused by:								
Rice weevil	44	80	93	88	94	41	95	76
Angoumois grain moth	56	20	7	12	6	59	5	24

[a] Includes the red flour beetle and the flat grain beetle.

by areas cannot be attributed to low temperature levels within areas as area number 3 contained as great or greater damage than in the State as a whole. Area 3 consisted of the north Louisiana hill country where the lowest temperatures of the State prevail. The authors believe that this difference in field infestation resulted indirectly from the cotton dusting program. Areas of least infestation occurred where cotton was concentrated and the cotton boll weevil poisoning program was most highly developed. The boll weevil control program in the

Table 3.—Infestation in corn by stored-grain insects. Louisiana, 1957–58.

	Areas of State							State Average
	1	2	3	4	5	6	7	
				Harvest, 1957				
Per cent ears infested by:								
Rice weevil	33	16	48	63	7	30	56	36
Angoumois grain moth	8	4	32	11	0	9	2	9
Square-necked grain beetle	57	82	84	89	85	75	95	81
Saw-toothed grain beetle	1−	1	1−	2	2	13	2	3
Per cent kernel damage	7	6	16	28	3	6	25	13
Per cent damage caused by:								
Rice weevil	99+	98	99+	99	100	98	99+	99
Angoumois grain moth	1−	2	1−	1	—	2	1−	1
				July, 1958				
Per cent ears infested by:								
Rice weevil	71	70	82	93	85	72	75	78
Angoumois grain moth	81	66	72	22	20	79	17	51
Square-necked grain beetle	39	61	45	65	80	28	86	58
Saw-toothed grain beetle	27	16	41	8	0	4	4	11
Per cent kernel damage	17	18	30	44	30	28	38	29
Per cent damage caused by:								
Rice weevil	72	88	87	100	100	80	100	90
Angoumois grain moth	28	12	13	0	0	20	0	10

Table 4.—Effect of shuck condition on infestation of corn at harvest by four species of stored-grain insects.[a]

Shuck Classification	Per Cent of Ears Infested by			
	Rice Weevil	Angoumois Grain Moth	Square-Necked Grain Beetle	Saw-Toothed Grain Beetle
Good	26	6	69	3
Poor	43	9	61	14

[a] Data from 2,726 ears with poor shuck condition and 3,625 ears with good shuck condition.

several years prior to 1955 contained BHC in the insecticide formulations. This chemical is extremely toxic to the rice weevil (Floyd & Newsom 1956). The peak of the cotton poisoning program coincided with the period of corn development when field infestation by the rice weevil occurs. It appears that sufficient drift of cotton poisons may have occurred which suppressed rice weevil development in corn fields interspersed among cotton fields. Much less BHC was used in the 1956 cotton insect control program as the cotton boll weevil was developing resistance to this insecticide. In 1957 practically none was used in the cotton insecticide formulations in Louisiana. Damage by the rice weevil in corn increased in these areas each year and approached the State average in 1957.

Data given in table 4 show the importance of good husk coverage. Almost twice as many ears with poor husk coverage were infested by rice weevils as were ears with good husk coverage. Ears with poor husk coverage were observed to be more heavily damaged than infested ears having good husk coverage. Difference in degree of infestation is an important factor in subsequent damage during storage. Importance of good husk coverage in protecting ears from infestation by Angoumois grain moths readily may be seen (table 4). However, quality of husk coverage in protection of the ear from this species is not as important as for the rice weevil. Infestation by the square-necked grain beetle apparently was not influenced by husk condition. This species was extremely abundant.

The saw-toothed grain beetle occurred most abundantly in ears with poor husk conditions. However, it is likely that this infestation probably did not originate in the field. The writers feel that most, if not all, of the saw-toothed grain beetles found in the fall examinations represented individuals that entered the ears after they were stored. Other studies conducted in Louisiana by the authors and associates show that only rarely is this species found in standing corn. Thousands of ears have been examined just previous to harvest but only two ears have been found infested with this species.

A few weeks after storage a high percentage of the square-necked grain beetles disappeared. It apparently was unable to maintain its original population status as the corn became drier in storage. Contrariwise the saw-toothed grain beetle appears to increase in numbers as the storage season advances.

As the storage season advanced and damage to the grain by insects increased, weight per bushel of corn was found to decrease in the face of moisture loss from an average of 57 pounds at harvest to 53 pounds the following summer.

References Cited

Floyd, E. H., and L. D. Newsom. 1956. Protection of stored corn with lindane-impregnated sawdust. Jour. Econ. Ent. 49(6): 753–7.

Floyd, E. H., J. D. Powell, and J. W. Ingram. 1958. Some factors influencing the infestation in corn in the field by the rice weevil. Jour. Econ. Ent. 51(1): 23–26.

Powell, J. D. 1958. The maximum grain moisture of field corn at which the rice weevil can develop. Masters' thesis—Louisiana State University.

Table 5.—Insect damage[a]—Weight per bushel relationship.

Area	Season of the Year									Total No. Ears Represented
	At Harvest			May			July			
	Kernel Damage (%)	Weight per Bushel (lb.)	Grain Moisture (%)	Kernel Damage (%)	Weight per Bushel (lb.)	Grain Moisture (%)	Kernel Damage (%)	Weight per Bushel (lb.)	Grain Moisture (%)	
1	3.5	58.0	15.6	2.0	58.0	13.2	15.4	55.9	12.4	1,060
2	4.5	57.9	13.8	9.0	56.3	13.1	19.1	56.2	12.2	1,520
6	4.4	58.1	14.7	10.5	55.1	13.0	29.4	52.9	12.2	1,480
4	20.8	55.6	14.8	28.7	52.5	13.3	33.0	51.2	12.6	1,380
7	19.1	56.7	14.0	26.9	53.5	12.8	34.4	52.2	12.1	1,940
5	9.4	57.4	—	27.1	54.4	13.3	39.0	51.0	12.9	1,120
3	12.7	55.9	15.1	15.8	53.3	13.2	42.9	51.0	12.7	1,260
State Average	10.6	57.1	14.7	17.1	54.7	13.1	30.5	52.9	12.4	

[a] Includes damage by the rice weevil and the Angoumois grain moth

Toxicity of Several Insecticides to Two Species of Beneficial Insects on Cotton[1]

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ABSTRACT

Laboratory tests conducted at College Station, Texas, indicated that dieldrin was the least toxic material tested against *Hippodamia convergens* G.-M. by a petri-dish method and was followed in order of increasing toxicity by endrin, BHC, toxaphene-DDT, dieldrin-DDT, heptachlor-DDT, heptachlor, DDT, parathion and methyl parathion. When applied topically, dieldrin was again the least toxic material, followed in order of increasing toxicity by toxaphene, DDT, and endrin.

Toxaphene was the least toxic material tested against adult *Orius insidiosus* (Say) by confining the insects to treated plants for 2 days, and was followed in order of increasing toxicity by DDT, dieldrin, heptachlor, aldrin, malathion, methyl parathion, and parathion. Toxaphene-DDT was the least toxic of the five insecticide mixtures tested.

Laboratory tests were conducted at College Station, Texas, to determine the relative contact toxicity of several widely used insecticides to the convergent lady beetle, *Hippodamia convergens* G.-M., and the insidious flower bug, *Orius insidiosus* (Say). These two species of insects are considered important predators of certain cotton pests throughout the central Texas area (Iglinsky & Rainwater 1950, Fletcher & Thomas 1943, Ewing & Ivy 1943).

Several investigators have reported results of field experiments regarding the toxicity of insecticides to predaceous insects on cotton; however, little work of this nature has been conducted in the laboratory. From the results of laboratory tests, Harries & Valcarce (1955) concluded that dust formulations of the phosphorus compounds parathion, malathion, Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O*,*O*-dimethyl phosphorothioate) and Diazinon® (*O*,*O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiate), were significantly more toxic to the convergent lady beetle than any of the chlorinated hydrocarbon dust formulations tested. Campbell & Hutchins (1952), working with dust and spray formulations, found that the convergent lady beetle was relatively tolerant to many of the insecticides tested.

Only spray formulations of insecticides were tested in the present study as dusts are not commonly used for cotton insect control in this local area.

MATERIALS AND METHODS.—*Hippodamia convergens.*—The beetles used in these tests were collected from alfalfa fields with an insect net. Two methods of treatment were employed, the petri-dish method and topical application.

In the petri-dish method the bottom of the lower half of each petri dish was covered with a disk cut from a large cotton leaf. Each leaf disk was the same diameter as the inside of the petri dish and fitted snugly against the bottom of the dish. Covers for these containers were made from screen wire. The dishes, with the leaf sections in place, were sprayed in a metal cylinder (18 inches in diameter by 44 inches in height). The screen covers were placed upside down beside the petri dishes in the cylinder so that they too received an application of insecticide. The insecticidal sprays, consisting of emulsifiable concentrates diluted with water, were delivered through a hole in the top of the cylinder with a solid cone nozzle at a pressure of 40 p.s.i. After spraying, the containers were removed from the chamber and the beetles introduced into them. The insects were allowed to remain on the treated surface for 4 hours and then were removed and confined to clean plastic refrigerator dishes (1.5 inches in depth by 3.5 inches in diameter) for the remainder of the 5-day testing period. All of the tests were conducted at room temperatures ranging from 75° to 95° F. Cotton aphids were supplied as food for the beetles on the second and fourth day. The mortality was recorded every 24 hours throughout the 5-day period. Percentages of mortality were calculated by Abbott's formula. Seven beetles were used in each replicate. Three dosage levels of each insecticide were used and each dosage was replicated a total of either 10, 15, or 20 times. A dosage-mortality curve was calculated for each insecticide.

The technique described above was employed to test only the initial contact toxicity of the insecticides. Since the screen covers of the containers, as well as the leaves, were treated, all the beetles were in contact with a treated surface for a like period of time; therefore, the results obtained by the use of this method were fairly consistent.

For the topical applications the technical grade insecticides were dissolved in acetone and applied to the dorsal suture between the prothoracic and mesothoracic segments of weighed beetles. The applications were made with a 0.25 ml. tuberculin syringe equipped with a 26-gauge needle and actuated by a micrometer. After treatment, the beetles were held in plastic dishes as described above for the petri-dish method. Mortality records were made 3 days after application of the insecticides. Data from four to six dosages of each insecticide were used in the calculation of each dosage-mortality curve. Each dosage was replicated at least 4 and not more than 16 times using 10 beetles in each replicate.

Orius insidiosus.—Adults of this species used in these tests were collected with an insect net from flowering alfalfa fields. These insects were especially abundant in the fields during the late spring and early summer months.

A technique somewhat similar to that used by Campbell & Hutchins (1952) on several species of predaceous insects was employed to determine the effect of the insecticides on this insect. This technique consisted of confining the insects on treated cotton terminals bearing two or three small leaves. The cotton cuttings were kept fresh during the 2-day testing period by placing the cut end of each stem in a bottle of nutrient solution buried

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Table 1.—Median lethal dosages of several insecticides tested against adult *Hippodamia convergens*.

Testing Method and Insecticide Used	Number Insects in Test	LD_{50}	Slope of Dosage-Mortality Line
Petri-dish method[a]			
Dieldrin	330	2.768 ± .303	2.93
Endrin	315	.846 ± .109	2.54
BHC	340	.621 ± .022	9.26
Toxaphene-DDT (2-1)	420	.319 ± .029	3.46
Dieldrin-DDT (1-2)	350	.268 ± .026	3.27
Heptachlor-DDT (1-2)	315	.222 ± .027	2.69
Heptachlor	315	.199 ± .012	5.55
DDT	350	.086 ± .014	1.94
Parathion	365	.003 ± .0004	2.70
Methyl parathion	280	.002 ± .0004	1.63
Topical application[b]			
Dieldrin	480	1.596 ± .373	1.15
Toxaphene	620	1.069 ± .149	1.35
DDT	680	.584 ± .108	1.24
Endrin	160	.106 ± .026	2.18

[a] Median lethal dosages expressed in pounds of active ingredients per acre.
[b] Median lethal dosages expressed in milligrams insecticide per gram body weight.

in a small sand-filled flower pot. Lantern globes were used as cages to confine the insects to the treated plants. A square of white cloth with a small hole in the center for the stem of the plant to pass through, was placed over each sand-filled pot. This cloth faciliated the counting of the dead insects which fell from the plant and also prevented the escape of the small insects by forming a tight seal around the bottom of the lantern globe. A hole was cut in the center of the cloth top of each lantern globe and was stoppered with a No. 10 cork stopper. It was through this hole that the flower bugs and their prey were introduced after the plants were sprayed and the lantern globes set in place.

The cotton cuttings were sprayed in a cylinder as described above for *Hippodamia convergens*. The sprays consisted of emulsifiable concentrates of the insecticides diluted with water.

Seven to 15 insects were used in each replicate and each dosage was replicated a total of eight times. From three to five dosages were used to calculate each dosage-mortality curve. Cotton aphids or spider mites were provided as food for the flower bugs on the first and second day of the tests. Mortality records were made 2 days after confining the bugs to the treated plants. All tests were conducted at room temperatures which ranged from 85° to 90° F.

Table 2.—Median lethal dosages of several insecticides tested against adult *Orius insidiosus*.

Insecticide	Number Insects in Test	LD_{50}[a]	Slope of Dosage-Mortality Line
Toxaphene	456	0.145 ± .022	2.072
DDT	433	.038 ± .011	1.661
Dieldrin	265	.017 ± .002	2.622
Heptachlor	278	.017 ± .003	1.938
Aldrin	235	.012 ± .001	2.733
Malathion	419	.011 ± .003	1.137
Methyl parathion	303	.010 ± .001	2.706
Parathion	316	.005 ± .001	2.002

[a] Median lethal dosages expressed in pounds of active ingredients per acre.

Table 3.—Toxicity of five insecticide mixtures to adult *Orius insidiosus*.[a]

Insecticide	Active Ingredient (Lb./A.)	Per Cent Mortality
Toxaphene-DDT (2-1)	0.030	53.3
	.090	86.4
Malathion-DDT (1-2)	.015	43.3
	.045	64.8
Dieldrin-DDT (1-2)	.015	60.0
	.045	94.5
Heptachlor-DDT (1-2)	.015	53.3
	.045	100.0
BHC-DDT (0.9–1.5)	.0015	60.0

[a] A total of 40 insects were tested at each dosage level.

RESULTS.—As may be noted in tables 1, 2, and 3, the concentrations, excluding the topical applications, required to obtain median lethal dosages are much lower than would normally be applied in the field. In the present study, these concentrations are expressed on pounds of active ingredients per acre basis merely as a matter of convenience in calculation and application. Since this investigation was conducted in the laboratory and was designed to test only the relative toxicities of the insecticides, such high rates of mortality should not necessarily be expected from similar low dosages under field conditions.

Hippodamia convergens.—The results of these tests are presented in table 1. The insecticides are arranged under each of the two testing methods in the order of increasing toxicity to the beetles. Dieldrin was the least toxic material tested by either method. Endrin, while being one of the least toxic materials tested by the petri-dish method, was the most toxic of four insecticides applied topically. The three mixtures, toxaphene-DDT, dieldrin-DDT and heptachlor-DDT, were of about equal toxicity to the convergent lady beetle.

Orius insidiosus.—The median lethal dosages of eight insecticides tested against this species are presented in table 2. These insecticides are arranged according to increasing toxicity to this species. Toxaphene was the least toxic material tested, while the two phosphorus insecticides malathion and methyl parathion were only slightly more toxic than either dieldrin, heptachlor, or aldrin.

The results of tests with five insecticide mixtures are presented in table 3. On the basis of these data, toxaphene-DDT was the least toxic, followed in order of increasing toxicity by malathion-DDT, either heptachlor-DDT or dieldrin-DDT, and BHC-DDT. Because of the small number of insects used here, more data are needed to draw definite conclusions as to the relative toxicity of these insecticide mixtures.

REFERENCES CITED

Campbell, William V., and Ross E. Hutchins, 1952. Toxicity of insecticides to some predaceous insects on cotton. Jour. Econ. Ent. **45**(5): 828–33.

Ewing, K. P., and E. E. Ivy, 1943. Some factors influencing bollworm populations and damage. Jour. Econ. Ent. **36**(4): 602–6.

Fletcher, R. K., and F. L. Thomas, 1943. Natural control of eggs and first instar larvae of *Heliothis armigera*. Jour. Econ. Ent. **36**(4): 557–60.

Harries, F. H., and A. C. Valcarce, 1955. Laboratory tests of the effect of insecticides on some beneficial insects. Jour. Econ. Ent. **48**(5): 614.

Iglinsky, William, Jr., and C. F. Rainwater. 1950. *Orius insidiosus* an enemy of a spider mite on cotton. Jour. Econ. Ent. **43**(4): 567.

Branch "Tip" Sampling for Determining Abundance of Spruce Budworm Egg Masses[1]

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ABSTRACT

Counting of the spruce budworm (*Choristoneura fumiferana* (Clem.)) on balsam fir branches has always been a tedious task. A technique is presented which modifies previously developed methods by reducing the size of the sample without greatly decreasing its accuracy. The technique consists essentially of separating the tips of the shoots from the rest of the branch. The number of egg masses on the tips must be increased by only 16% to obtain the total number on the branch. Time saved by this technique ranges from 25 to 40%, depending upon the size of the branch, over previous techniques. Branches of any size and from any level of the crown are within the scope of this technique. The method is not feasible on branches with severe or complete defoliation.

Conventional methods for sampling egg masses of the spruce budworm (*Choristoneura fumiferana* (Clem.)) on balsam fir and spruce trees have proved to be reliable for the comparison of various areas for survey purposes. The use of a 15-inch sample, taken from the apical terminus of a branch in the midcrown, was developed by Dowden & Carolin (1950). Similarly an 18-inch sample used by Atwood (1944) to determine the number of mined needles was an adequate sampling unit for most egg-mass surveys. At times, when greater accuracy is desired, the entire branch must be used to eliminate the error that would result from the comparison of 15- or 18-inch samples taken from different sized branches from different crown levels.

Counting egg masses on entire branches or on numerous 15- or 18-inch samples is a tedious and often a costly operation. Morris (1955) contends that no system of partial sampling is feasible. However, he based his conclusion solely on an age stratification. A study recently conducted in northern Minnesota indicates that a partial sample based on area stratification is reliable.

MATERIALS AND METHODS.—The foliage used in this study was collected from 14 widely separated areas on the Superior National Forest in northern Minnesota. Many of the samples consisted of 15-inch twigs cut from the termini of midcrown branches. The remaining samples were whole branches of various lengths clipped from the top, mid, and basal third of each tree's crown.

In the first part of the experiment 15 branches were chosen at random, and the position of each egg mass was plotted on a drawing of each branch. The length of the branchlet and twig, the degree of defoliation of each twig, and the distance of each egg mass from the end of the twig were recorded. From these data it appeared that an appreciable amount of foliage could be discarded before inspection without greatly decreasing the accuracy of the sample.

This technique, which consisted essentially of separating the shoots from the rest of the branch, was then tested on 145 samples. Each branch or 15-inch twig was separated into two sampling universes consisting of (1) linear increments of foliage cut from the twigs which were termed "tips," and (2) the remaining foliage. The egg masses were counted on each sampling universe, and correlation and regression analyses were made.

In the following year, the validity of the technique was further tested with 112 branches.

RESULTS AND DISCUSSION.—A balsam fir or spruce branch is generally shaped like an isosceles triangle (figure 1A). Most branches over 1 year in age possess nodal branchlets (figure 1A, a) and numerous internodal branchlets (figure 1A, c, d). Several twigs or shoots are borne on the branchlets.

The results of the test showed that the egg masses were confined to a distinct area. Most of the masses on the lateral twigs were within 3 inches of the end of the shoots. On the apical shoots of the main stem (figure 1A, b), the egg masses were found within the last 4 inches of the end. Those egg masses that were laid on the internodal branchlets were nearly always confined to the apical half. If the shoots were partially defoliated the masses were still found within the 3-inch increment behind the point of defoliation.

Thus most of the masses were located on the "tips." Even when there were high numbers of egg masses on the "tips" the number on the remaining material was small. The fact that these remaining egg masses were usually in clusters suggested that they were the product of a single aberrant female.

The regression of the number of egg masses from the whole branch on the number of egg masses from the "tips" showed that an excellent correlation exists between the two factors (fig. 2). There are only 16% more egg masses on the whole branch than on the "tips."

On all but severely defoliated branches the egg masses are restricted to the shoot terminus, regardless of the size of the branch and its position on the tree. If the branch is

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[2] Maintained at St. Paul 1, Minn., by the Forest Service, U. S. Department of Agriculture, in cooperation with the University of Minnesota.

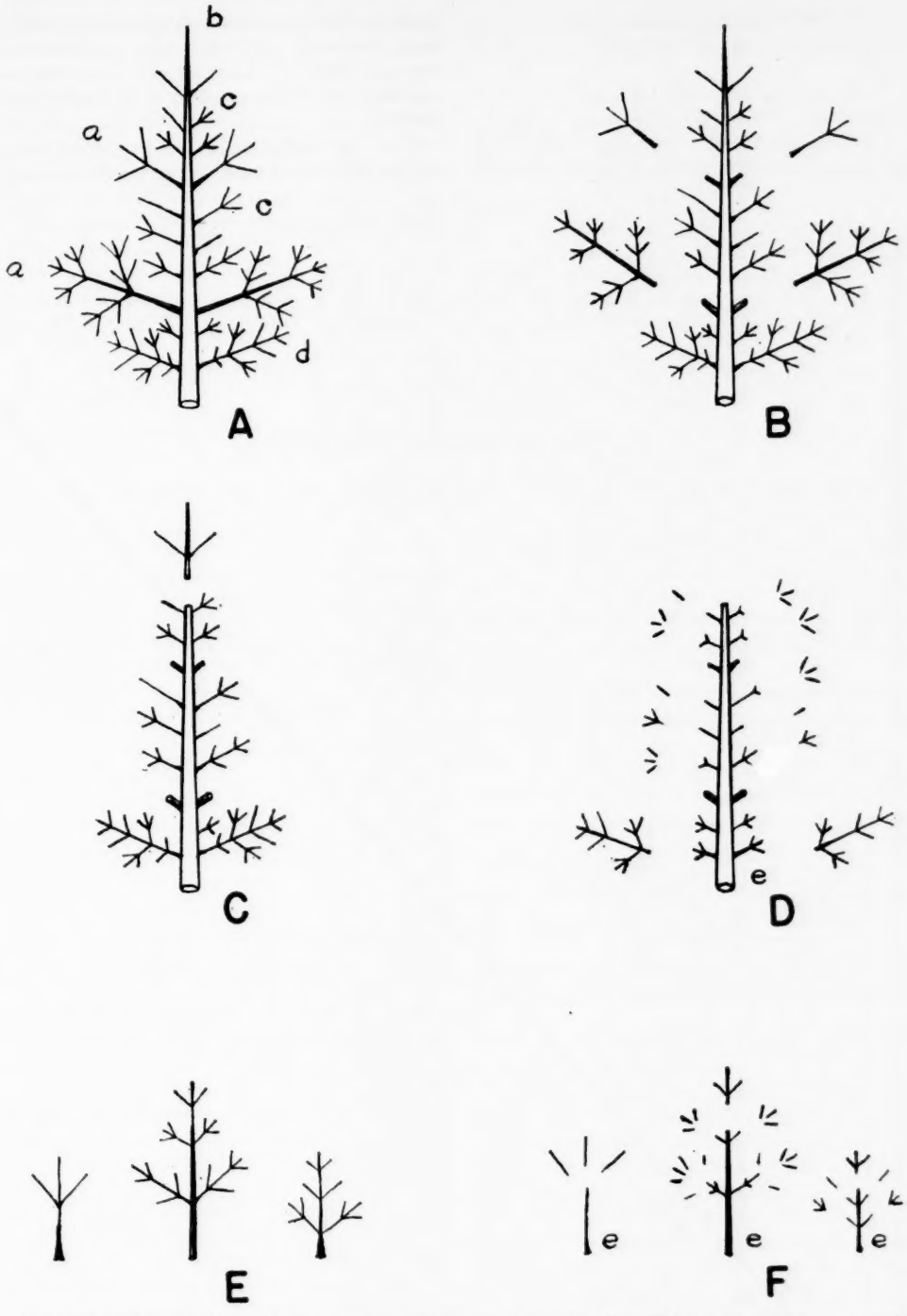

FIG. 1.—Branch "tip" sampling technique showing the proper sequence of sampling. Small letters in drawings A, D, and F denote the following: a, nodal branchlet; b, apical branch tip; c, short internodal branchlets; d, long internodal branchlets; e, discard material.

A. Cut balsam fir branch.

B. Cut nodal branchlets from main stem and put aside for further treatment.

C. If terminal shoot of main stem is not defoliated, cut it 4 inches behind bud; if defoliated, cut 4 inches behind defoliated area. Put aside for counting egg masses.

D. Cut short internodal branchlets (1 to 5 inches long) at their center and put aside for counting. Cut long internodal branchlets in same way as nodal branchlets and put aside for further treatment. Discard remaining (e) foliage.

E. Assemble nodal and long internodal branchlets.

F. Cut apical twigs from nodal and internodal branchlets; cut lateral twigs (less than 3 inches) at their base; cut lateral twigs (more than 3 inches) at their center. Put aside for counting egg masses. Discard remaining (e) foliage.

lightly or moderately defoliated the egg masses will be farther back on the shoots but still within 3 inches of the defoliated area.

However, if the branch is so severely defoliated that the remaining foliage is restricted to the branch stem, the egg masses will be found there. Occasionally defoliation is so complete that the moths have been observed to oviposit on the bare twigs and then to migrate to new locations. Severely and completely defoliated branches cannot be considered as material within the scope of this technique since the amount of foliage is considerably reduced.

The "tip" sampling technique was devised primarily to hasten the tedious job of egg-mass counting. In the old

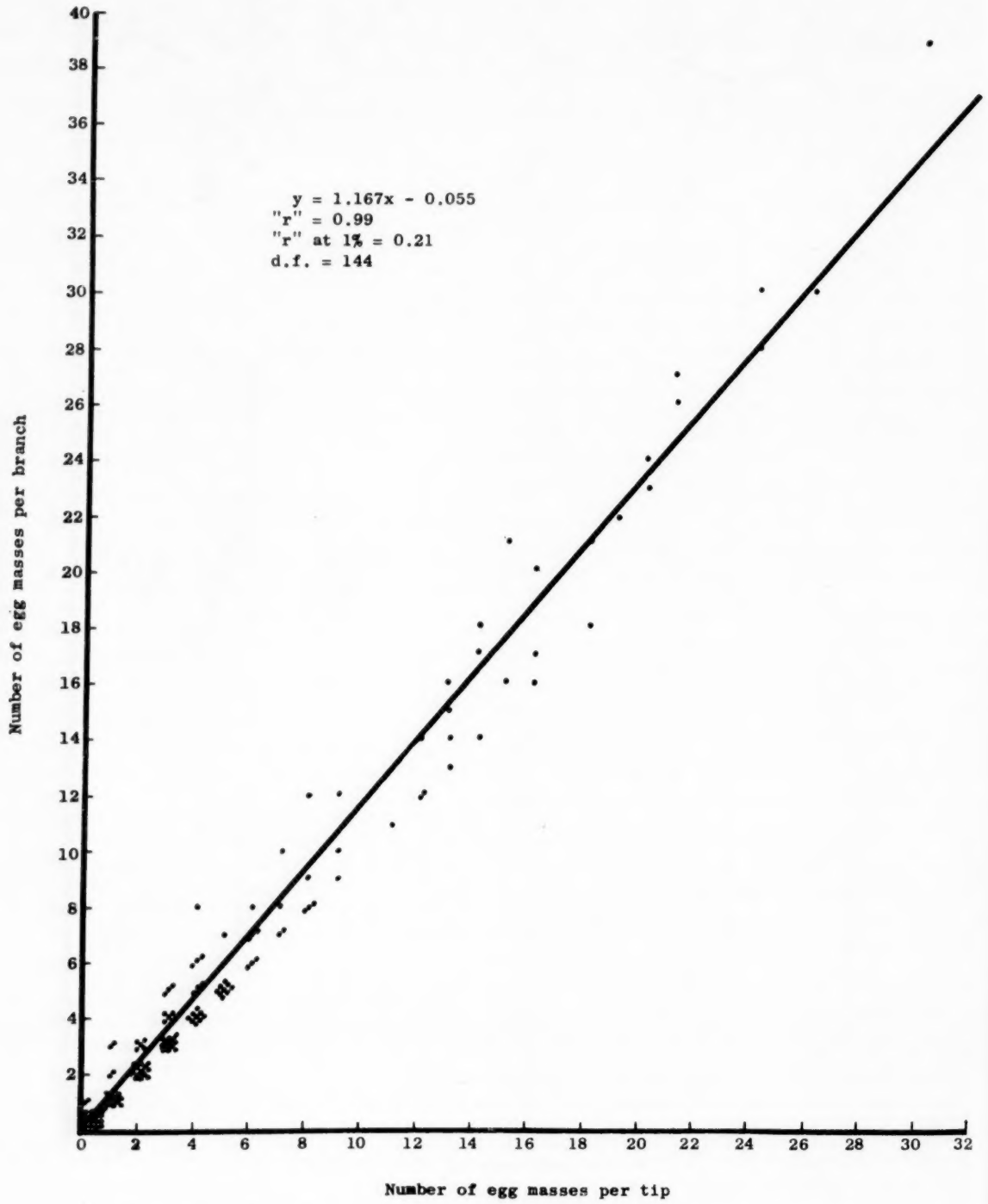

FIG. 2.—The number of egg masses per tip in relation to the number of egg masses per branch.

method the branches must be cut up into 2- or 3-inch lengths to facilitate egg mass detection by the investigator. This new technique differs only by selectively removing the essential material from the sample. The remaining foliage, which consists of about 35 to 40% of the total foliage on a relatively large branch and 25 to 30% on 15- or 18-inch twig samples, can be discarded in this technique. The amount of time gained is about equal to the amount of discard material. Both become proportionately less with increased defoliation.

BRANCH "TIP" SAMPLING TECHNIQUE.—The procedure for separating the tip material from the rest of the foliage is described step-by-step in figure 1. The egg masses on the tips are then counted and the total is increased by 16% to account for the egg masses on the discarded foliage.

REFERENCES CITED

Atwood, C. E. 1944. The feeding habits of the young spruce budworm larvae. Canadian Ent. **76**: 64–66.

Dowden, P. B., and V. M. Carolin. 1950. Natural control factors affecting the spruce budworm in the Adirondacks during 1946–1948. Jour. Econ. Ent. **43**: 774–83.

Morris, R. F. 1955. The development of sampling techniques for forest insect defoliators, with particular reference to spruce budworm. Canadian Jour. Zool. **33**: 225–94.

A Modified Olfactometer[1]

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ABSTRACT

This olfactometer differs from previous models in the materials used in construction and in technical modifications. Plastic tubing and lucite panels were used whenever possible. Two lucite cylinders connected by a small doorway form the insect test cage. This improvement makes the cage equal in size and shape to the influent ports, thus eliminating dead air spaces and the intermixing of the airflow between the alternative compartments.

Other improvements include accessibility of all the control mechanisms, modified influent ports, a double 3-way valve mechanism for shunting the airflow from one alternative compartment to the other, and the incorporation of hygrothermographic equipment onto a removable platform beneath the test cage.

In the past 50 years, olfactometer design has undergone considerable modification. Change was brought about partially through the advancement of technical equipment and materials, and partially through necessity. Even at the present time there is no olfactometer that works equally well with all species of terrestrial arthropods. As each new species was tested, investigators realized that changes were necessary. This is not to say that the earlier models were not effective; some of them are still being used. However, later models are generally more flexible.

The Y-tube principle, tested by Barrows (1907), proved to be reliable for his studies. McIndoo (1926) constructed an apparatus on the same principle, and with slight modifications this device is frequently used today. A few years later Hoskins & Craig (1934) designed a more versatile model, incorporating more regulatory equipment and modifying the apparatus to accommodate flying insects. However, the screen test cage had inadequate airflow properties and was in direct contact with two cylindrical influent ports. Wieting & Hoskins (1939) refined this design by making it more compact, and adding a 3-way valve for shunting the odor from one influent port to the other. Willis (1947), working with flying insects, changed the design still further by incorporating a part of the apparatus into a double-walled box to maintain a constant temperature. Realizing the shortcomings of the large-size test cage, which contained numerous dead air spaces, Willis & Roth (1950) altered it to a cylinder to conform with the size of the combined influent ports. Other improvements included temperature-control refinements.

The olfactometer described in this paper is a modification of the one developed by Willis & Roth (1950). Graduate students in entomology at the University of Minnesota have recently tested it with various species of arthropods. Satisfactory reactions were obtained with the granary weevil (*Sitophilus granarius* (L.)) and the rice weevil (*S. oryze* (L.)) in tests where a choice between fresh and beetle-infested grain was provided. The Angoumois grain moth (*Sitotroga cerealella* (Olivier)) and the confused flour beetle (*Tribolium confusum* Duval) reacted favorably to odors from moldy and fresh grain. The wood louse (*Cylisticus convexus* (De Geer)) showed rapid and pronounced reactions to various relative humidity conditions.

For a detailed explanation of the uses of an olfactometer see Dethier (1947).

DESCRIPTION OF APPARATUS.—The apparatus[3] is contained in a double-walled box with a removable light-tight cover. The three influent and one exfluent glass flowmeters are mounted between the walls behind a clear lucite panel to prevent breakage (fig. 1). All controls are attached to the exterior wall within easy reach of the operator. Previous models had some of the controls on the interior of the apparatus; this was both inconvenient and undesirable in that the cover should be left *in situ* when temperature and light conditions must be controlled.

The layout of the interior is essentially the same as that designed by Willis & Roth, but modifications are incorporated in the materials used and in construction (fig. 2). The glass connections are replaced by plastic (Pygon) tubing, and the observation chamber (fig. 3),

[1] Accepted for publication January 19, 1959.

[2] Maintained at St. Paul 1, Minn., by the Forest Service, U. S. Department of Agriculture, in cooperation with the University of Minnesota.

[3] The olfactometer was constructed by the Scientific Apparatus Services, University of Minnesota, under the direction of Mr. L. M. Espy, manager.

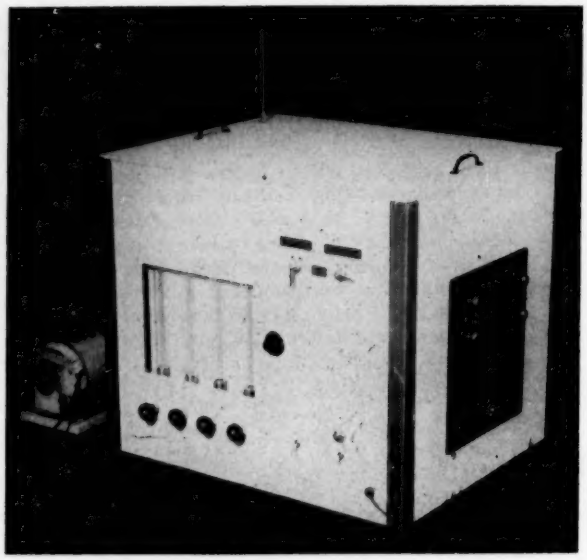

FIG. 1.—Olfactometer showing pump, flowmeters, valves, and exterior of the observation chamber.

including the platforms, is constructed entirely of lucite. The lucite sides and bottom are opaque; the top is transparent. The parts are bonded together with plastic adhesive or brass screws.

The cylindrical influent ports, which are made from clear lucite, are attached to the transparent top surface of the observation chamber. Air enters the port through a small opening (fig. 3, *A*). A perforated plate in the center of each port contains a mushroom-like projection (*B*), which deflects the incoming air. Smoke tests showed that this projection aided materially in the uniformity of the low-velocity air-flow through the test cage below.

The upper half of the observation chamber holds the test cage and the hygrothermographic equipment. The insect test cage, which consists of two lucite cylinders connected by a small doorway (fig. 4), is mounted on a

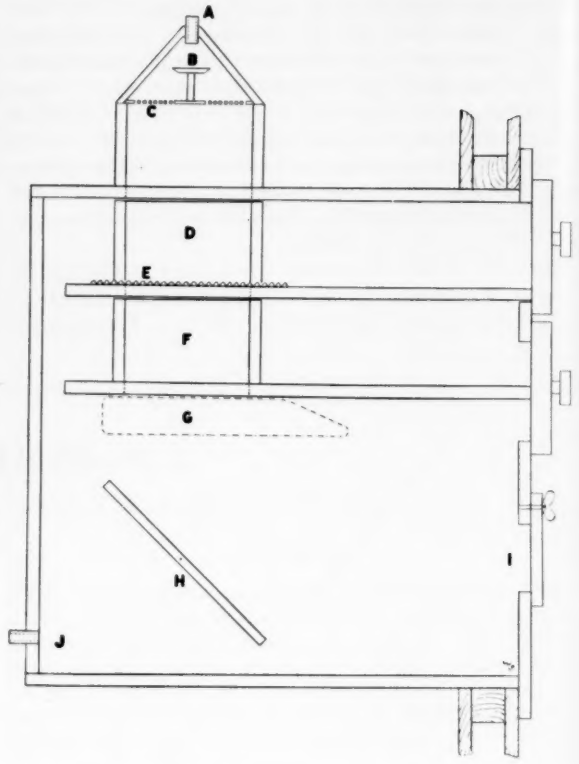

FIG. 3.—Diagram of the observation chamber. *A*, opening to influent port; *B*, plastic "mushroom" for dispersing airflow; *C*, plastic disc perforated with 290 $\frac{1}{16}$-inch holes arranged in 8 concentric rows; *D*, insect test cage on upper platform; *E*, screen floor of insect cage (a second screen disc is suspended about 1 inch above this screen when flying and climbing insects are tested); *F*, chamber for hygrothermographic equipment on lower platform; *G*, ramp along the walls to hold lower platform against the upper; *H*, mirror; *I*, observation or photographic window with sliding cover; *J*, exhaust port. Drawing is to scale: both the width and height of the chamber are 12 inches.

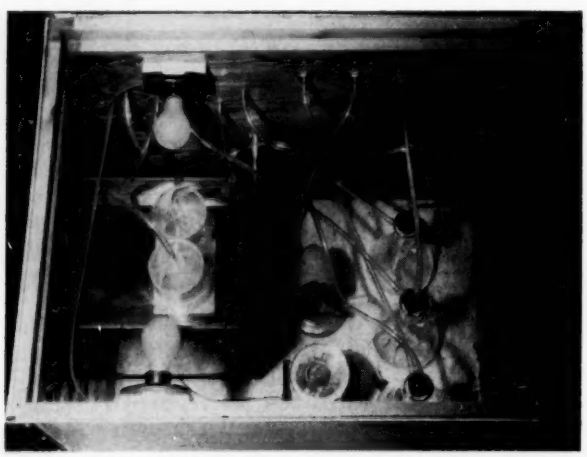

FIG. 2.—Olfactometer with cover removed showing observation chamber with influent ports, light and temperature regulating apparatus, plastic tubing, and odor vessels. (Front of apparatus is at the top of picture.)

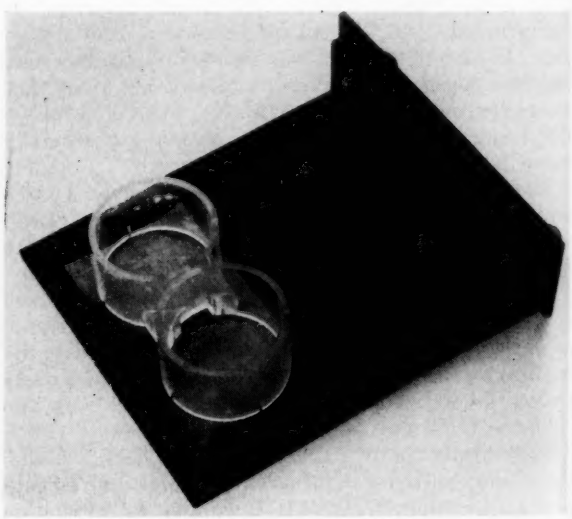

FIG. 4.—Platform with test cage composed of two lucite cylinders connected by a small doorway.

removable upper platform. These cylinders conform exactly with the size and shape of the combined influent ports (fig. 3, *D*). This modification eliminates the dead air spaces of previous test cages, and prevents mixing of the air between the two compartments.

Immediately below, also mounted on a removable platform, are two identical cylinders which carry the hygrothermographic equipment (*F*). When the two platforms are in place, each cylinder interlocks with the one above and make one long continuous cylinder.

The mirror, observation window, and exhaust port remain in a position similar to the equipment of Willis & Roth (fig. 3, *H*, *I*, *J*).

OPERATIONAL DESIGN.—Figure 5 shows the operation of the olfactometer. Air is pumped into the olfactometer by a low pressure pump (*A*), where it is sent through plastic tubing (*B*), and is immediately purified by passing through chunk bone-charcoal (*C*). If desired, the air may be humidified prior to its entry into the flowmeters by passing it through a vessel containing an appropriate acid or salt solution (*D*). The air then goes through a manifold attached to the three influent flowmeters, where its velocity is measured and regulated, and enters one or more vessels where it picks up the desired odor or odors to be tested. Figure 5 shows a setup designed for tests with only one odor. The vessel containing the odor in this instance is inserted into the airflow following the second flowmeter (*F*). If two different odors are to be compared simultaneously, two additional vessels are inserted into the airflow following the first and third flowmeters. The

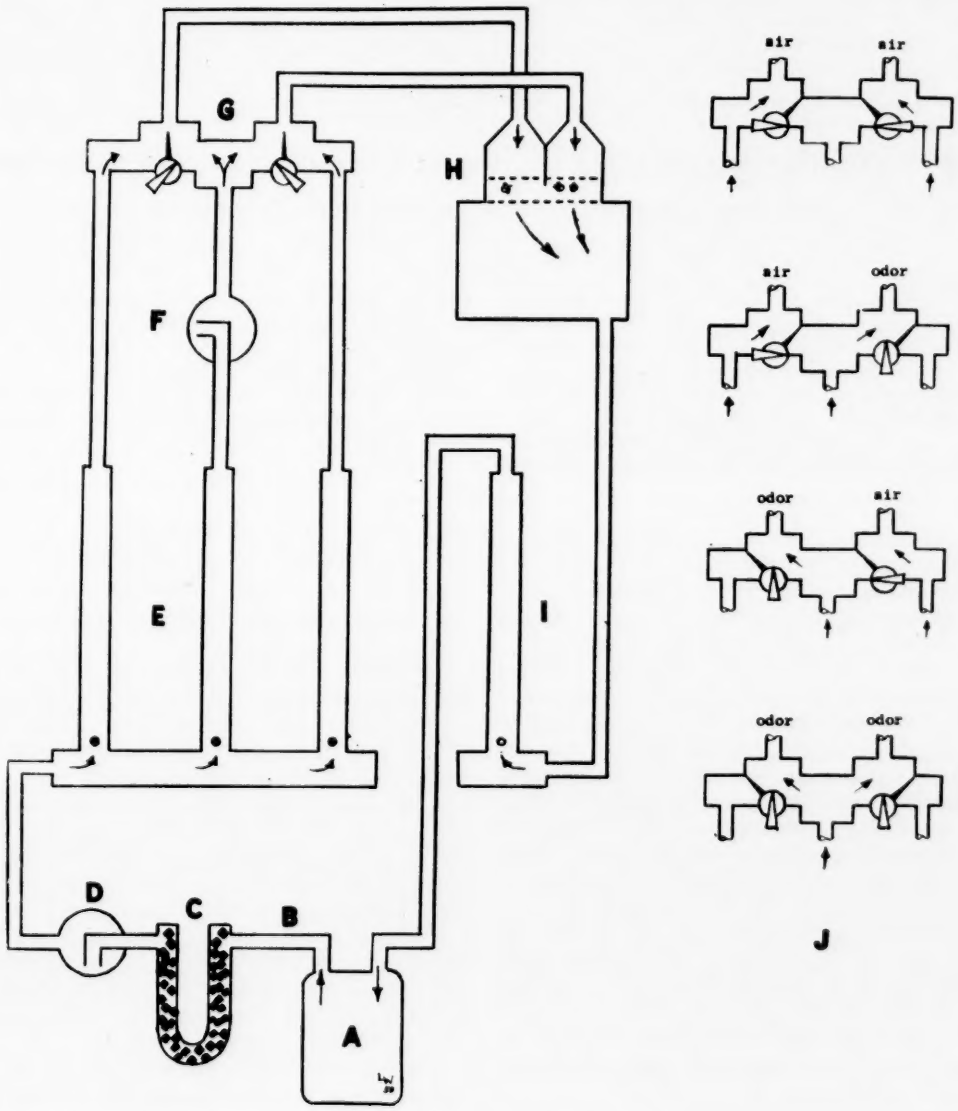

FIG. 5.—Schematic diagram of olfactometer. *A*, continuous circulating air pump attached to ¼ horsepower electric motor; *B*, plastic (Pygon) tubing; *C*, U-tube with chunk bone-charcoal; *D*, humidity regulating vessel; *E*, influents flowmeter; *F*, odor vessel; *G*, valve mechanism for shunting airflow; *H*, observation chamber; *I*, exhaust flowmeter; *J*, diagrammatic valves showing possible airflow combinations.

alternate odor is placed in both of these vessels. In experiments dealing with relative humidity instead of odor, the vessels contain the proper acid or salt solution. The container with the humidifying agent (*D*) in this instance would be eliminated. The valve mechanism (*G*) shunts the air to the influent ports above the test cage (*H*). After passing through the observation chamber the exhaust air is drawn through another flowmeter (*I*) back to the pump. This exfluent flowmeter has a steel indicator ball which is twice the weight of the sapphire balls of the other flowmeters. This is necessary to accommodate the air coming from two influent flowmeters at once. The double-valve mechanism used here allows four airflow combinations (*J*).

REFERENCES CITED

Barrows, W. M. 1907. The reactions of the pomace fly *Drosophila ampelophila* Loew. to odorous substances. Jour. Exptl. Zool. **4**: 515–37.

Dethier, V. G. 1947. Chemical insect attractants and repellents. The Blakiston Co. Phil., 289 pp.

Hoskins, W. M., and R. Craig. 1934. The olfactory responses of flies in a new type of insect olfactometer. I. Theory and design of the olfactometer. Jour. Econ. Ent. **28**: 1029–36.

McIndoo, N. E. 1926. An insect olfactometer. Jour. Econ. Ent. **19**: 545–71.

Wieting, J. O. G., and W. M. Hoskins. 1939. The olfactory responses of flies in a new type of insect olfactometer. II. Responses of the housefly to ammonia, carbon dioxide, and ethyl alcohol. Jour. Econ. Ent. **32**: 24–29.

Willis, E. R. 1947. The olfactory responses of female mosquitoes. Jour. Econ. Ent. **40**: 769–78.

Willis, E. R., and L. M. Roth. 1950. Humidity reactions of *Tribolium castaneum* (Herbst). Jour. Exptl. Zool. **115**: 561–87.

Aphid Populations on Oats Grown in Various Nutrient Solutions[1]

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ABSTRACT

Plant nutrient availability to Garry oats (*Avena sativa* L.) var. Garry was controlled by nutrient solutions in the greenhouse. The plants were infested by apple grain aphids, *Rhopalosiphum fitchii* (Sand.), according to their preference for individual plants and allowed to feed and reproduce thereon for a period of 14 days. The data indicated that there was a significantly greater population increase potential where nitrogen was available to the plants. Elimination of nitrogen from the nutrient solutions resulted in lower aphid populations and adversely affected the plants, as indicated by decreased plant height, yellowish coloration, and spindly growth. Aphid population on all treatments correlated with plant height to a highly significant degree. Whether population differences reported here were due primarily to nutrient media or plant height (leaf area) has not been determined.

Several authors have reported the effect of mineral-deficient plants on insect development and injury. Inconsistent results point to the need to investigate each crop-insect association individually, for a more uniform procedure and for a uniform criteria of evaluation.

Haseman (1946) used nutrient solutions to vary mineral availability to wheat and found that the rate of of greenbug (*Toxoptera graminum* (Rond.)) development was slowed down when nitrogen was withheld from the plants. This work is in closer agreement with the results reported here than where minerals were applied to soil in the field or greenhouse. Arant & Jones (1951) varied the amount of nitrogen applied to the soil and reported fewer greenbugs per square centimeter of oat leaf with increased application of nitrogen to the soil. Blickenstaff *et al.* (1954) reported less greenbug damage on oats with increased application of nitrogen to the soil. Daniels (1957) reported fewer greenbugs per gram of leaf and less damage on wheat when nitrogen was applied to soil in greenhouse tests. Greenbugs were most numerous in the field on heaviest applications of nitrogen, but lowest when measured per gram of foliage.

While studying the development of hessian fly, *Phytophaga destructor* (Say), populations on wheat grown in nutrient solutions of various compositions in the greenhouse, apple grain aphids, *Rhopalosiphum fitchii* (Sand.), infested the test plants. The aphid populations were observed to vary in size depending on the treatment to which the plants were subjected. These observations were explored in more detail in the experiments outlined below.

MATERIALS AND METHODS.—All work was done in a greenhouse at 65 to 70° F. and approximately 65% relative humidity. Oats (*Avena sativa* L.) var. Garry was grown in nutrient solutions on special plant trays. The apple grain aphid was the test insect.

Eight plant trays were constructed from $\frac{3}{4}$-inch plywood stock according to the measurements given in figure 1. Four groupings of $\frac{1}{2}$-inch diameter holes were drilled through each board. The under side was covered with 16-mesh plastic screen.

Each plant tray was placed on top of four 1-pint polyethylene freezer containers (fig. 1) which held the nutrient solutions. Each container under a tray held a different solution and each treatment was replicated four times with four plants at each location.

The plant trays were prepared by placing a thin layer of vermiculite in each hole, seeding in two seeds of Garry

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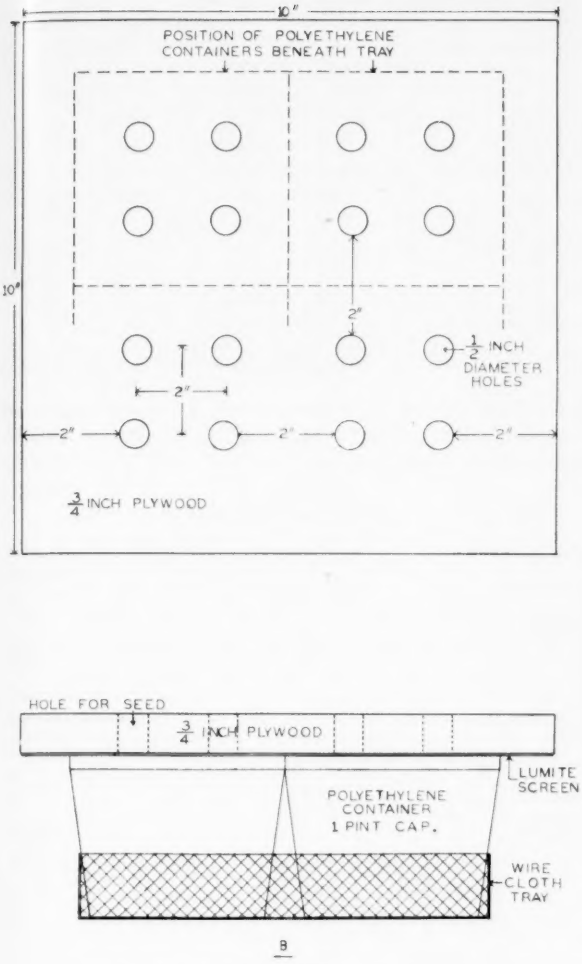

FIG. 1.—Details for construction of plant trays.

oats, and filling the hole with additional vermiculite. Each tray was placed over four empty polyethylene containers, the vermiculite watered and then covered with wet paper toweling. Excess water dripped into the containers to provide a saturated humidity in the area of future root growth. Three to 4 days after seeding top growth appeared. The paper toweling was removed at this time and the vermiculite kept wet with gentle surface watering. When root growth was about 1 inch in length the plastic containers were filled with 500 ml. of tap water. This provided the liquid growth medium for several days and forced stored food depletion in the seed.

The nutrient solutions were patterned after Hoagland & Arnon (1950). Stock solutions of each salt, prepared according to table 1, were diluted with tap water in the volumes indicated to prepare the various nutrient solutions. Iron and a supplemental solution of minor elements were added to each complete mixture.

Nutrient solutions replaced the water 9 days after seeding and were replaced with fresh solutions after 12 days' use. The nutrient solutions were not aerated.

Plant height was recorded by measuring the length of the longest leaf. Aphid populations were recorded by counting the number of aphids in all stages of maturity on each plant.

When plants were 9 days old, they were thinned to 1 plant per hole. Aphids that had infested the plants were removed and the number of aphids per plant recorded at intervals thereafter. These counts reflected plant attractiveness plus the reproductive capacity of adults on plants of each treatment.

RESULTS.—Aphid population, plant height, and number of leaves per plant are recorded in table 2. Analysis of variance showed that aphid populations on the various treatments were not significantly different 16 days after planting, or during the first 7 days after initial infestation. During the first 4 days of infestation there was a gradual increase in aphid numbers with not much spread in population between treatments. On the seventh day increases in aphid population were more noticeable in treatments containing nitrogen.

Greenhouse records have revealed that approximately 7 days are required for a full life cycle of the apple grain aphid. Fourteen days after initial infestation, the population of aphids per plant was reflecting the influence of the host upon reproductive rate and preference under greenhouse conditions. At this point, analysis of variance indicated significant differences in aphid populations among treatments at the 1% level of probability and no significant differences among replications. The least significant differences between two means at the 5% and 1% levels (table 2) showed that with the exception of the comparison NK:P, all treatments containing nitrogen had higher

Table 1.—Composition of nutrient solutions and p.p.m. of each element in the final solution.

Nutrient Solution	Salt	Concentration of Stock Solution		Ml. Stock Solution per 2 Liters of Nutrient Solution	Nutrient Elements[a] (p.p.m.)
NPK	KH_2PO_4		Molar	2	N=210
	KNO_3		Molar	10	P= 31
	$Ca(NO_3)_2$		Molar	10	K=234
	$MgSO_4$		Molar	3	
NP	$Ca(NO_3)_2$		Molar	15	N=210
	$Ca(H_2PO_4)_2$	.05	Molar	20	P= 31
	$MgSO_4$		Molar	3	
NK	$Ca(NO_3)_2$		Molar	9	N=210
	KNO_3		Molar	12	K=234
	$MgSO_4$		Molar	3	
PK	K_2SO_4	.5	Molar	12	P= 31
	$Ca(H_2PO_4)_2$	.05	Molar	20	K=234
	$MgSO_4$		Molar	3	
	$CaSO_4$	.01	Molar	400	
N	$Ca(NO_3)_2$		Molar	15	N=210
	$MgSO_4$		Molar	3	
P	$Ca(H_2PO_4)_2$	.05	Molar	20	P= 31
	$MgSO_4$		Molar	3	
	$CaSO_4$	.01	Molar	400	
K	K_2SO_4	.5	Molar	12	K=234
	$MgSO_4$		Molar	3	
	$CaSO_4$	.01	Molar	400	
Fe[b]	Iron tartrate	.05%	Soln.	2	
Supplement[b]	H_3BO_3	2.86	gm./l.		B= 0.5
	$MnCl_2 \cdot H_2O$	1.81	gm./l.		Mn= 0.5
	$ZnSO_4 \cdot 7H_2O$	0.22	gm./l.	2	Zn= 0.05
	$CuSO_4 \cdot 5H_2O$	0.08	gm./l.		Cu= 0.02
	$M_2MoO_4 \cdot H_2O$	0.02	gm./l.		Mo= 0.01

[a] Total p.p.m. from all salts used in preparing the final nutrient solution.
[b] These solutions were added to each of the nutrient solutions to supply iron and minor elements.

Table 2.—The influence of nutrient availability on aphid population and plant growth.

Age of Plant (days)	Days After Infestation	Average Number of Aphids per Plant								L.S.D.	
		NPK	NP	NK	PK	N	P	K	H_2O	.05	.01
10	1	2.3	1.6	2.1	1.7	2.2	2.0	3.2	1.2	1.8	2.4
11	2	3.2	3.2	2.1	2.2	3.0	3.1	3.0	1.9	2.1	2.9
13	4	4.8	3.5	3.2	2.6	4.1	4.7	4.8	3.8	3.3	4.5
16	7	11.2	12.1	7.0	4.0	6.2	9.4	10.6	4.1	6.5	8.8
23	14	43.3	43.0	35.0	14.4	38.8	20.4	15.0	15.0	17.9	24.4
	Germination	Average plant height (cm.)									
10	10	8.9	10.4	11.0	9.0	10.9	10.2	8.7	9.0	3.5	4.8
17	17	17.4	15.7	16.1	10.7	14.2	11.7	10.9	10.7	4.9	6.7
24	24	21.4	18.8	20.4	12.4	17.2	12.7	13.8	12.5	3.9	5.4
		Number of leaves per plant									
32	32	4.6	4.3	4.5	4.5	4.3	4.0	4.4	4.3	0.6	0.9

populations per plant than nitrogen deficient media. Plants growing in tap water responded as in a nitrogen deficient medium. Nitrogen appeared to be the major controlling element with phosphorus and potassium of minor importance.

Nitrogen availability influenced plant height (table 2) to a significant degree after 17 days of growth. This influence was magnified with increasing days. Plants growing without nitrogen were yellowish in color, shorter and more spindly than plants with nitrogen available. A correlation between plant height and aphid population on all treatments at three dates showed no correlation 1 day following infestation, but highly significant correlations 7 ($r = .8640$) and 14 ($r = .9104$) days later. The number of leaves per plant between treatments was not significantly different.

These data do not separate the influence of nutrient media and plant height or leaf area upon the aphid populations. The significant differences recorded here may have been caused by either or both factors. Data from experiments designed to interpret this association will be presented in a later paper.

References Cited

Arant, F. S., and C. M. Jones. 1951. Influence of lime and nitrogenous fertilizers on populations of greenbugs infesting oats. Jour. Econ. Ent. **44:** 121–2.

Blickenstaff, C. C., D. D. Morey, and G. W. Burton. 1954. Effect of rates of nitrogen application on greenbug damage to oats, rye, and ryegrass. Agron. Jour. **46:** 338.

Daniels, N. E. 1957. Greenbug populations and their damage to winter wheat as affected by fertilizer applications. Jour. Econ. Ent. **50:** 793–4.

Haseman, L. 1946. Influence of soil minerals on insects. Jour. Econ. Ent. **39:** 8–11.

Hoagland, D. R., and D. I. Arnon. 1950. The water-culture method for growing plants without soil. California Agric. Expt. Sta. Circ. **347.**

Fluorinated Analogs of DDT as Toxicants and DDT Synergists[1]

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Abstract

The insecticidal activities and ability to synergize DDT of 16 fluorine-containing structural analogs of DDT were determined.

Of the 2,2-difluoro-1,1-di-*p*-halophenyl derivatives of ethane, ethanol and ethyl acetate, the *p*-bromo- and *p*-chloro- compounds were about equally toxic to susceptible house flies (*Musca domestica* L.) and the *p*-fluoro-compounds were less toxic. The ethane, ethanol, and ethyl acetate derivatives decreased in toxicity in the order given. The fluorodiphenylethenes were not significantly toxic to house flies.

Maximum synergistic activity with DDT occurred in those derivatives containing *p*-chloro- or bromo-groups, fluoro- being inferior in all cases. Synergistic activity varied with the nature of the group attached to the alpha carbon atom in the following order: $OH > H > OCOCH_3$. The fluorodiphenylethenes were the least active *p*-halophenyl synergists. The difluorodiphenyl compounds containing no halogen substituent in the benzene ring were devoid of synergistic activity.

The possibility of controlling DDT-resistant insects with compounds structurally related to DDT was emphasized by Summerford *et al.* (1951). They reported that 1,1-bis-(*p*-chlorophenyl)ethanol (DMC) and its 4,4′-difluoro-analog appreciably increased the toxicity of DDT to resistant house flies. Others (March *et al.* 1952; Speroni 1952; Tahori 1955; Nieman *et al.* 1956) have shown that the most effective synergists of DDT are structurally related to it.

Tahori (1955) demonstrated that *p*-chloro- and *p*-bromo- substituted 2,2,2-trifluoro-1,1-diphenylethanols,

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ethanes and acetates are effective DDT synergists. Tahori found that 2,2,2-trifluoro-1,1-bis(*p*-chlorophenyl) ethanol was two to five times more effective than the corresponding non-fluorinated carbinol (DMC), and 1.5 times more effective than 1,1-bis(*p*-chlorophenyl)ethane.

In this study *p*-substituted 2,2-difluoro-1,1-diphenylethanols, their corresponding acetates and ethanes, and the monofluoroethenes were prepared (Bornstein *et al.* 1957) and tested for toxicity to susceptible house flies (*Musca domestica* L.) and for synergistic activity with DDT on resistant house flies.

EXPERIMENTAL.—The house flies used in this investigation were the National Association of Insecticide and Disinfectant Manufacturer's 1948 susceptible strain and the Orlando-Beltsville DDT-resistant strain. The larvae were reared on moist Purina dog pellets in a room held at 32° C.±1° and 50% R.H.±5%. Except for the period during which they were being treated with the test chemicals, adults were held at the same conditions and fed a water suspension of powdered milk. Adult females, 3 to 4 days old, were lightly anesthetized with carbon dioxide and treated topically with 1 μl. of an acetone solution of the test compound delivered from a micrometer driven syringe. Each compound was tested on 4 samples of 20 flies from each of 2 populations. When the test compounds were evaluated as synergists, they were mixed with DDT at the ratio of 5 parts DDT to 1 part test compound. Six dose levels of this mixture were applied. Dosage-mortality curves were obtained by plotting the toxicity data on log probability paper. LD 50 values were computed by slope determination of the response curves.

RESULTS AND DISCUSSION.—An LD 50 for DDT could not be obtained on resistant flies since 120 μg./fly was the maximum amount practicable to deliver in 1 μl. of acetone. None of the analogs caused over 5% mortality to resistant flies when applied at 10 μg./fly.

The toxicities to house flies of the compounds alone and in combination with DDT are listed in table 1. Combinations of DDT and the test compounds were evaluated only on resistant flies since synergism of DDT has only been demonstrated with resistant strains (March *et al.* 1952).

The most toxic compounds to susceptible flies were those containing *p*-chloro- or *p*-bromo- substituents (table 1). In the 2,2-difluoro-1,1-di-*p*-halophenyl derivatives of ethane, ethanol and ethyl acetate, the *p*-chloro- and bromo- compounds had similar toxicities (1,2,3,5,6,7). The substitution of *p*-fluoro- groups (9,10,11) reduced toxicity when compared to chloro- and bromo-. The unsubstituted diphenyl derivatives (13,14,15,16) were not significantly toxic.

In the three *p*-halogen series, ethanols, ethanes, and ethyl acetates, the nature of the group attached to the alpha carbon atom affected insecticidal activity. The most toxic compounds within any halogen series were the ethanes (1,5,9). When the alpha hydrogen was replaced by hydroxyl to give the ethanol, toxicity was reduced (2,6,10). Toxicity decreased when the alpha hydroxyl group was acetylated (3,7,11). The ethenes (4,8,12) prepared by the dehydrofluorination of the ethanes had little toxicity.

Maximum synergistic activity with DDT generally occurred with the *p*-chloro- and *p*-bromo-substituted compounds, which were similar in activity. The *p*-fluoro- and the unsubstituted diphenyl derivatives were less active. The activities of the well known DDT synergists 1,1-bis-(*p*-chlorophenyl)ethanol (DMC) (17), 1,1-bis-(*p*-chloro-

Table 1.—Toxicity of analogs of DDT to susceptible house flies and DDT plus the analogs at a 5:1 ratio to resistant house flies.

ANALOG	FORMULA	SUSCEPTIBLE	RESISTANT
		Analog LD-50 (μg/fly)[a]	DDT Present in Mixture at LD-50 (μg/fly)[b]
1. 2,2-Difluoro-1,1-bis-(*p*-chlorophenyl)ethane	$(p\text{-}ClC_6H_4)_2CHCHF_2$	2.5	0.70
2. 2,2-Difluoro-1,1-bis-(*p*-chlorophenyl)ethanol	$(p\text{-}ClC_6H_4)_2COHCHF_2$	3.7	0.62
3. 2,2-Difluoro-1,1-bis-(*p*-chlorophenyl)ethyl acetate	$(p\text{-}ClC_6H_4)_2C(O_2CCH_3)CHF_2$	9.2	1.60
4. 2-Fluoro-1,1-bis-(*p*-chlorophenyl)ethene	$(p\text{-}ClC_6H_4)_2C{=}CHF$	>10 (18)	11.90
5. 2,2-Difluoro-1,1-bis-(*p*-bromophenyl)ethane	$(p\text{-}BrC_6H_4)_2CHCHF_2$	2.4	0.96
6. 2,2-Difluoro-1,1-bis-(*p*-bromophenyl)ethanol	$(p\text{-}BrC_6H_4)_2COHCHF_2$	4.8	0.71
7. 2,2-Difluoro-1,1-bis-(*p*-bromophenyl)ethyl acetate	$(p\text{-}BrC_6H_4)_2C(O_2CCH_3)CHF_2$	9.3	1.70
8. 2-Fluoro-1,1-bis-(*p*-bromophenyl)ethene	$(p\text{-}BrC_6H_4)_2C{=}CHF$	>10 (18)	7.20
9. 2,2-Difluoro-1,1-bis-(*p*-fluorophenyl)ethane	$(p\text{-}FC_6H_4)_2CHCHF_2$	4.8	7.60
10. 2,2-Difluoro-1,1-bis-(*p*-fluorophenyl)ethanol	$(p\text{-}FC_6H_4)_2COHCHF_2$	6.7	3.48
11. 2,2-Difluoro-1,1-bis-(*p*-fluorophenyl)ethyl acetate	$(p\text{-}FC_6H_4)_2C(O_2CCH_3)CHF_2$	>10 (36)	8.50
12. 2-Fluoro-1,1-bis-(*p*-fluorophenyl)ethene	$(p\text{-}FC_6H_4)_2C{=}CHF$	>10 (17)	>12 (29)
13. 2,2-Difluoro-1,1-diphenylethane	$(C_6H_5)_2CHCHF_2$	>10 (24)	>12 (17)
14. 2,2-Difluoro-1,1-diphenylethanol	$(C_6H_5)_2COHCHF_2$	>10 (12)	>12 (24)
15. 2,2-Difluoro-1,1-diphenylethyl acetate	$(C_6H_5)_2C(O_2CCH_3)CHF_2$	>10 (12)	>12 (10)
16. 2-Fluoro-1,1-diphenylethene	$(C_6H_5)_2C{=}CHF$	>10 (22)	>12 (8)
17. 1,1-bis-(*p*-chlorophenyl)ethanol (DMC)	$(p\text{-}ClC_6H_4)_2COHCH_3$	—	3.70
18. 1,1-bis-(*p*-chlorophenyl)ethane	$(p\text{-}ClC_6H_4)_2CHCH_3$	—	1.60
19. 1,1-bis-(*p*-chlorophenyl)ethene	$(p\text{-}ClC_6H_4)_2C{=}CH_2$	—	4.4
20. 1,1-bis-(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT)	$(p\text{-}ClC_6H_4)_2CHCCl_3$	0.35	—
21. Acetone		1 μl (3±2)	1 μl (3±2)

[a] 10 μg. was maximum dose applied, resulting mortality in parentheses.
[b] 12 μg. was maximum dose applied, resulting mortality in parentheses.

phenyl)ethane (18) and 1,1-bis-(*p*-chlorophenyl)ethene (19) are listed for comparison.

The most active synergists were 2,2-difluoro-1,1-bis-(*p*-chlorophenyl)ethanol and 2,2-difluoro-1,1-bis-(*p*-bromophenyl)ethanol (2,6). Both of these compounds were superior to the non-fluorinated 1,1-bis-(*p*-chlorophenyl) ethanol, DMC (17). The *p*-chloro- and *p*-bromo-substituted diphenylethanes (1,5) were less active than the corresponding ethanols (2,6), but more active than 1,1-bis-(*p*-chlorophenyl)ethane (18). 2,2-difluoro-1,1-bis-(*p*-fluorophenyl)ethanol (10) is about one-fifth as active as the analogous chloro- and bromo-derivatives (2,6). This compound was slightly more effective than DMC (17) and was about one-half as active as 1,1-bis-(*p*-chlorophenyl) ethane (18). The *p*-fluoro- substituted ethane (9) was approximately one-tenth as active as the corresponding chloro- derivative (1) and the least active of the *p*-halophenylethanes. Furthermore, this compound was less than half as active as the corresponding ethanol (10).

The 2,2-difluoro-1,1-bis-(*p*-halophenyl)ethyl acetates showed the same activity pattern as the ethanols and ethanes, the *p*-chloro- (3) and *p*-bromo- (7) derivatives both being more active than the *p*-fluoro-compound (11). The chloro- and bromo- acetates (3,7) were about half as active as the parent ethanols (2,6) but were more active than DMC (17) or 1,1-bis-(*p*-chlorophenyl)ethane (18). The *p*-fluoro- substituted acetate (11) was about one-fifth as active as the corresponding chloro- and bromo-derivatives (3,7).

The *p*-halo-substituted diphenylethenes (4,8,12) were less active than the ethanols (2,6,10), acetates (3,7,11) and ethanes (1,5,9), from which they were derived. 2-Fluoro-1,1-bis-(*p*-chlorophenyl)ethene (4) was less active than 1,1-bis-(*p*-chlorophenyl)ethene (19). The *p*-fluoro-derivative (12) was considerably less active than the corresponding chloro- and bromo- compounds (4,8).

The insecticidal activities of these compounds containing fluorine on the terminal carbon atom resemble those found in DDT and its derivatives (containing chlorine on the terminal carbon). The most toxic compounds were the ethanes, whereas the corresponding ethanols were less active. Similarly, DDT is more toxic than its corresponding ethanol (Reuter & Ascher 1956). When the 2,2-difluoro-1,1-diphenylethanes are dehydrofluorinated, the resulting ethenes are almost devoid of insecticial activity. This also follows the pattern of DDT, which is more active than its dehydrochlorinated analog (Sternberg *et al.* 1950).

Tahori (1955) evaluated a series of *p*-substituted 2,2,2-trifluoro-1,1-diphenylethanes, ethanols and ethyl acetates against *Musca vicina* Macq. He reported the *p*-chloro- and *p*-bromo-ethanes and ethanols to be of similar toxicities. However, in contrast to our results with diphenylethyl acetates, he reported that the trifluorodiphenylethyl acetates were similar in toxicity to the ethanes and ethanols. It is interesting to note that our series of ethenes had no significant toxicity, and were the least active synergistically. This was also the case with the acetates, which were less toxic and less active synergistically than the ethanes and ethanols.

In the *p*-chloro- and *p*-bromo-substituted difluorodiphenylethanols, a slight decrease in synergistic activity occurred when the hydroxyl group was replaced by hydrogen. This structure-activity correlation was more marked in the corresponding *p*-fluoro-substituted derivatives, where replacement of hydroxyl by hydrogen resulted in a large decrease in synergistic activity.

The similar synergistic activities of the various *p*-chloro- and *p*-bromo- derivatives within the same series is striking. In the ethanes, ethanols and acetates, the compounds containing *p*-chloro- or *p*-bromo- substituents were nearly equal in synergistic activity in every case. The fact that both chloro- or bromo- were equally good *p*-substituents contrasts with the decreased synergistic activity observed with the corresponding compounds carrying the smaller fluorine atom in the *p*-position.

Acetylation of the various ethanols resulted in a decrease in synergistic activity. It is possible that these acetates are not hydrolyzed rapidly by the house fly, for if they were, they would be expected to be as active as the ethanols.

The increase in synergistic activity obtained when fluorine is substituted for hydrogen on the terminal alkyl carbon is pronounced. For example, 2,2-difluoro substitution of 1,1-bis(*p*-chlorophenyl)ethanol (DMC)(17) and its corresponding ethane (18) increases synergistic activity. Tahori (1955) has reported a similar increase in synergistic activity with 2,2,2-trifluoro-substitution.

Fluorination of the side chain of 1,1-bis(*p*-chlorophenyl)ethene resulted in a decrease in synergistic activity (4,19). Similarly, March *et al.* (1952) reported that replacement of one side chain hydrogen by chlorine in the *p*-chloro- substituted diphenylethene series resulted in a decrease in synergistic activity. Consequently, it is evident that side chain halogenation of 1,1-bis-(*p*-chlorophenyl)ethene reduces synergistic actity.

The results of Tahori (1955), who evaluated the synergistic activities of trifluorodiphenylethanols, ethanes and acetates, were similar to those herein reported for the corresponding difluoro-compounds.

REFERENCES CITED

Bornstein, J., M. S. Blum, and J. J. Pratt, Jr. 1957. DDT synergists. The synthesis and properties of some 2,2-difluoro-1,1-diarylethanols and 2-fluoro-1,1-diarylethenes. Jour. Organ. Chem. **22:** 1210–13.

March, R. B., R. L. Metcalf, and L. L. Lewallen. 1952. Synergists for DDT against insecticide resistant house flies. Jour. Econ. Ent. **45:** 851–60.

Nieman, M., A. Modiano, G. G. Mer, and R. Cevlich. 1956. Substituted benzenesulphonanilides as synergists for DDT. Nature **177:** 800–1.

Reuter, S., and K. R. S. Ascher. 1956. The action of di-(*p*-chlorophenyl)-trifluoromethylcarbinol on house flies. Experienta **12:** 316–18.

Speroni, G. 1952. Impiego de sinergici nell lotta contro la. *Musca domestica* resistente agli insetticidi di contatto. La Chemica e l'industria. **34:** 391–403.

Sternburg, J., C. W. Kearns, and W. N. Bruce. 1950. Absorption and metabolism of DDT by resistant and susceptible house flies. Jour. Econ. Ent. **43:** 214–9.

Summerford, W. T., M. B. Geote, K. D. Quarterman and S. L. Schenk. 1951. The potentiation of DDT by several structurally related compounds. Science **114:** 6–7.

Tahori, A. S. 1955. Diaryl-trifluoromethylcarbinols as synergists for DDT against DDT-resistant house flies. Jour. Econ. Ent. **48:** 638–42.

Observations on Black Flies in Two Maryland Counties[1]

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ABSTRACT

In recent years residents of the Maryland suburbs of Washington, D. C., have been bothered by small non-biting flies that swarm around the head and enter the eyes, ears, nose, and mouth. It was known that some of the flies causing annoyance were black flies. Surveys were conducted during 1956 and 1957 to identify the annoying flies and to learn as much as possible about their ecology and distribution within Prince Georges and Montgomery Counties. In 1956, 215 observations were made on habits of adults of various species at 43 stations; 92 collections were made at 21 stations; 123 observations were negative. *Simulium jenningsi* Malloch adults were collected at 18 stations and were present at one or more stations during each 10-day period from May to November. There were 186 different observations on larvae at 40 stations during 1956 with collections made at 36 stations; 42 observations were negative. *S. jenningsi* larvae were collected at only five stations, four of which were located in the Potomac River. Additional observations in 1957 provided further evidence that *S. jenningsi* was the predominant species. Adults were common throughout the metropolitan areas of the two counties, but larvae were found almost exclusively in the Potomac River where they were associated with waterwillow, *Dianthera americana* L. It appears that there are two principal generations which usually emerge early in May and early in June.

Ten different species of black flies were collected during the surveys: *Prosimulium hirtipes* (Fries), *P. magnum* Dyar and Shannon, *Cnephia mutata* (Malloch), *Simulium fibrinflatum* Twinn, *S. venustum* Say, *S. parnassum* Malloch, *S. vittatum* Zetterstedt, *S. decorum* Walker, *S. jenningsi*, and *S. tuberosum* (Lundstroem). Adult Chloropidae and Drosophilidae were collected occasionally.

Adult females of *Simulium decorum*, *S. venustum*, and *S. parnassum* were recorded as biting man during this survey.

Various formulations of BHC, DDT, and malathion were used in fog machines, and formulations of BHC and DDT were used in mist blowers for control of adults. Unsatisfactory results were obtained in that relief from fly annoyance lasted for only a few hours probably because relatively small areas (500 acres) were treated.

In recent years small non-biting flies have been rather obnoxious to residents of the Maryland suburbs of Washington, D. C. The Department of Entomology of the University of Maryland has received many complaints about small flies or gnats which are troublesome primarily because they swarm around the head and enter the eyes, ears, nose, and mouth. There were occasional reports of small flies biting. It was known that some of the flies causing annoyance were black flies.

The present study was initiated (1) to identify the annoying flies or gnats, (2) to determine the geographic distribution within metropolitan Prince Georges and Montgomery Counties, (3) to locate sources of larvae, (4) to obtain qualitative information about larval habitats, (5) to obtain records of seasonal distribution, and (6) to conduct small-scale experiments on controlling adult populations with insecticides.

Dr. Alan Stone, of the U. S. Department of Agriculture (personal communication), indicated that the majority of the pestiferous flies could be expected to be black flies, members of the family Simuliidae. Accordingly, surveys were planned to investigate potential larval habitats, and this part of the work began in the spring of 1956. Surveys for larvae and/or adults were conducted from March to November 1956 and were continued during the spring and summer of 1957.

The more important contributions to knowledge of the biology and taxonomy of Nearctic Simuliidae are those of Malloch (1914), Twinn (1936), Underhill (1944), Nicholson & Mickel (1950), Stains & Knowlton (1943), Stone & Jamnback (1955), Jamnback (1956), and Sommerman *et al.* (1955). In addition to the papers listed above the treatise by Dalmat (1955) was referred to in planning and executing the work reported here.

SURVEY METHODS.—Collecting stations were selected with the idea of obtaining representative samples of larvae from typical streams throughout parts of the two Counties. The accompanying map shows the general location of 39 stations visited in 1956 and 1957. At the majority of stations attempts were made to collect both larvae and adults.

Stream beds were examined at what appeared to be favorable sites for black fly larvae and pupae. They were found attached to grasses and other plants, stones, sticks, and other debris. They were removed with forceps and preserved in vials of 70% alcohol. As was expected, larvae or pupae were found exclusively in rapidly flowing water. Larval populations in stream beds often fluctuated greatly within 1 or 2 square feet so that it was difficult to obtain an accurate estimate of the degree of infestation.

There was some variation in the area of stream bed included in a station or collecting site. In general, observations of stones and the like were made across the width of a shallow stream and at scattered points for a distance of 20 or 30 feet up and down the stream. At times explorations of stream beds extended up to several hundred yards and occasionally as much as one-half mile. An arbitrary system of recording the relative abundance of larvae or pupae was established. It was as follows:

None—No larvae found.
Trace—One or two larvae collected at the station.
Few—No difficulty was encountered in finding larvae, but they were few and scattered.
Medium—Several larvae present over the entire area.

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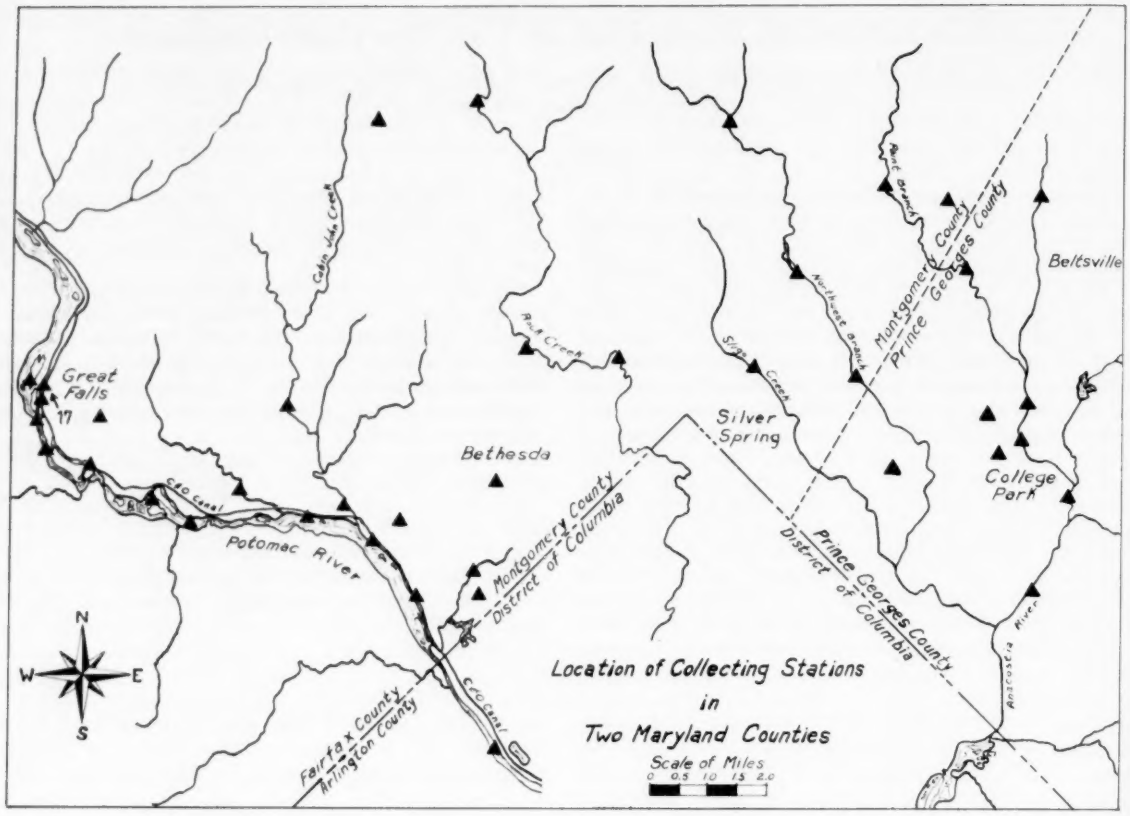

Heavy—A heavy concentration of larvae; many present over the entire area. Objects more or less covered with larvae.

Adult flies were usually collected by sweeping an insect net around the head of the collector or his helper. As the flies crawled up the side of the net while attempting to escape they were counted and forced into vials of 70% alcohol. By counting the number of flies in a sweep it was possible to determine a light, medium, or heavy infestation. One or two flies per sweep was considered a light infestation (referred to as few); two to six, a medium infestation; and more than six, heavy. The heavy infestation, however, was not recorded as such unless it lasted for at least 5 minutes.

Some of the stations were visited only once or twice; others were visited at weekly intervals from April to November.

RESULTS AND DISCUSSION.—In 1956, 215 observations were made on habits of adults of various species; 92 collections were made at 21 stations. In the early stages of the adult surveys it became obvious that *Simulium jenningsi* Malloch was the predominant species responsible for the annoyances previously mentioned. Adults of this species were taken at 12 stations during May, and they were abundant and annoying at several places. Throughout the summer of 1956 they were collected at 18 stations, and they were present at one or more stations during each 10-day period from May 11 through November 10.

There were 186 different observations on larvae during 1956, and collections were made at 36 stations. Larvae of *Simulium jenningsi* were collected at only five stations, four of which were located in the Potomac River; the fifth was very nearly a part of the River—a small watercourse by-passing Lock 18 of the Chesapeake and Ohio Canal just below Great Falls. Among other things the survey in 1956 indicated that there were large numbers of *S. jenningsi* larvae in the Potomac River and that larvae of this species were absent from the creeks and smaller streams sampled.

Simulium jenningsi luggeri was described as a subspecies of *S. jenningsi* Malloch by Nicholson & Mickel (1950). This subspecies can be separated from nominate *jenningsi* only in the pupal stage. Stone and Jamnback failed to find *S. jenningsi luggeri* in New York. A few *luggeri* pupae were collected in the Potomac River in April and May 1956 and 1957. In five cases the two pupae were found next to each other at the same station. This fact suggests that the two forms are not subspecies. It is possible that they should be treated as separate species. Additional information is needed to clarify the status of these taxa.

Because *Simulium jenningsi* was found to be more important than any other species in 1956, greater emphasis was placed on observing and collecting this species in 1957 than in 1956. More than 100 observations on adults of all species were made, and there were 45 collections, all but 6 of which consisted of *S. jenningsi*. Collections were somewhat irregular and scattered, but conclusive evidence was accumulated to show that *S. jenningsi* adults were distributed throughout the area studied, and that there were heavy populations at one or more stations from May through September.

More than 50 observations on larvae were made in 1957. Larvae and/or pupae of several species were found at 14 stations. Observations at Station 17 were intensified during 1957 because it was an ideal habitat for the immature stages of *Simulium jenningsi*. This station consisted of a small branch of the Potomac River at Great Falls between the dam and the falls proper. It was the most accessible of several small branches near the east bank which were separated by small islands. The current was rather swift and there were a few rapids. The width and depth varied with seasonal fluctuations in volume of water, but wading was nearly always possible.

Larvae of *S. jenningsi* began to appear early in May 1956 on moss, stones, sticks, and grass. Pupae were present on May 11, and by May 25 most adults of the first brood had emerged. By the end of May adults had been collected at 12 stations at distances from the river up to 11.5 miles. Meanwhile observations at larval stations in small streams throughout the study area failed to reveal the presence of immature stages of this species.

In the river on May 25, just after most adults of the first brood had emerged, small larvae were observed to be very abundant on leaves of young waterwillow, *Dianthera americana* L. Larvae were present on the waterwillow throughout the rest of the summer. Adults of the second brood seemed to emerge rather gradually. A third brood was not clearly indicated although another peak in the larval population occurred in early August. Adult populations were heavy at scattered stations until late August. Adults were sometimes annoying until the middle of October.

In 1957 larvae appeared in April, and pupae were seen on April 30. Some adults emerged on May 1. Larvae and pupae of the first brood were collected from waterwillow on May 6 when the plants were 3 to 4 inches high. Very few pupae were observed on May 16, suggesting that emergence of the first brood had ended. Small larvae of the second brood were present on May 16 and pupae appeared on May 22. Most larvae changed to pupae during a 4-day period ending May 30. Most adults of the 1957 second brood emerged during the first week in June. Population peaks were observed about 10 days earlier in 1957 than in 1956. The water level in the Potomac River during the summer of 1957 was much lower than in 1956 owing to a very dry season.

Larvae of the second brood occurred on the young waterwillow leaves that were barely submerged in rapidly moving water. Underhill (1944) reported that waterwillow in large streams in Virginia provided an ideal environment for larvae and pupae of *S. nigroparvum* Twinn, the species here referred to as *S. jenningsi* Malloch.

The term "brood" is used in discussing the biology of this black fly because there is insufficient evidence that population peaks represent different generations. It is thought that the two broods mentioned are two generations, but it is theoretically possible that the second larval population peak results from a delayed hatching of eggs deposited the previous year. Presumably many adults, soon after they emerge, disperse rather rapidly over many square miles in a few days. Underhill (1944) collected adults 20 to 30 miles from the nearest known breeding place and reported large numbers 10 miles from the nearest known breeding place. In view of Underhill's studies it does not seem unreasonable to assume that adults traveled to Beltsville, 13 miles from the Potomac. No information on oviposition of *S. jenningsi* was obtained in these studies. If the second "brood" is a true generation—the offspring of the first "brood"—then an important question remains unanswered. Do mating and oviposition take place before the dispersal of adults? An affirmative answer is suggested by some circumstantial evidence, namely, larvae of the second "brood" appeared very soon after adults of the first brood emerged. Perhaps some or many females travel several miles from the Potomac and return later to oviposit. At any rate they do not seem to oviposit elsewhere. Because waterwillow is known to grow rather profusely in the Potomac River for many miles above Great Falls, at least to a point near Hancock, it is assumed that tremendous numbers of *S. jenningsi* are produced each year.

Ten species of black flies in addition to *Simulium jenningsi* were collected during the period of these studies. *S. fibrinflatum* Twinn, *S. parnassum* Malloch, *Cnephia mutata* (Malloch), and *Prosimulium magnum* Dyar and Shannon appeared to be so rare in the two Maryland counties that no discussion is warranted. A single adult of *S. aureum* Fries was collected during 1958.

Larvae of *Simulium decorum* Walker were collected at five stations, well separated geographically. Adults of this species were collected several times during 1956 and 1957, and on one occasion a biting collection was made at Great Falls.

Larvae of *Simulium vittatum* Zetterstedt were taken almost every week from March to November 1956. They were at 17 stations at various times. Two peaks of larval abundance were noted, one in late May and the other in October. Adults of this species were collected infrequently in April, May and June.

From April to November 1956 larvae of *Simulium tuberosum* (Lundstroem) were collected from most of the permanent swift streams in the area surveyed. There were three peaks of larval abundance in late May, late June, and early September. No adults of this species were identified in any collections made. At one station *S. tuberosum* larvae were found heavily parasitized by nematodes of the genus *Paramermis*. Since these worms undoubtedly prevent larvae from maturing they play a part in natural control later in the season.

A few larvae of *Prosimulium hirtipes* (Fries) were collected in March and April 1956 and 1957. In April 1956 a few adults of this species were collected at three widely separated stations. At this time they were not biting, but this species is known to be a troublesome biter in other parts of Maryland.

Larvae of *Simulium venustum* Say were found in April and May 1956 and 1957 in small swift streams throughout the area surveyed. There is probably a single generation each year. Between late April and early June adults were collected at seven widely separated stations. Only two bona fide biting records were obtained. This species is an important biting pest at many places in North America. In the areas surveyed, adults were never abundant.

As a result of the adult surveys it was concluded that the vast majority of flies which cause annoyance by swarming around the face are *Simulium jenningsi*. Occasionally there were collections of flies other than black

flies. Two species of *Hippelates* (Family Chloropidae) were collected a few times. There were 27 collections of 1, 2, or 3 species of *Amiota* (Family Drosophilidae). These were of relatively little importance.

CONTROL INVESTIGATIONS.—Tests using fog machines were conducted in both 1957 and 1958 for adult black fly control. Several tests were made in residential areas, and others were conducted at a 500-acre country club. Fogs made from mixtures of 2.7% lindane in No. 2 fuel oil and 1.3% and 1.5% lindane in Mobilsol "F"[3]; or a mixture of 3.1% malathion in Mobilsol "F"; or mixtures of 10% and 12% DDT in Mobilsol "F" failed to produce satisfactory relief from the flies.

Two attempts at control in 1957 using a large mist blower likewise gave no noticeable relief. The first formulation consisted of 6 gallons of 25% DDT and 4 gallons of BHC (11% gamma) in 90 gallons of water, and the second formulation was a 1.2% lindane mixture. Unsatisfactory results were obtained, *i.e.*, relief from fly annoyance lasted only a few hours. This was attributed to the fact that only relatively small areas (not more than a 500-acre area) were treated.

REFERENCES CITED

Dalmat, H. T. 1955. The black flies (Diptera:Simuliidae) of Guatemala and their role as vectors of onchocerciasis. Smithsonian Misc. Coll. **125**(1): 1–425.

Jamnback, H. A. 1956. An illustrated key to the black fly larvae commonly collected in New York State. New York State Science Service. Albany. 10 pp.

Malloch, J. R. 1914. American black flies or buffalo gnats. U. S. Dept. Agric. Bur. Ent. Tech. Ser. **26**: 1–74.

Nicholson, H. P., and C. E. Mickel. 1950. The black flies of Minnesota. Univ. Minnesota Agric. Expt. Sta. Tech. Bull. **192**: 1–64.

Sommerman, K. M., R. I. Sailer, and C. O. Esselbaugh. 1955. Biology of Alaskan black flies (Simuliidae, Diptera). Ecol. Monogr. **25**: 345–85.

Stains, G. S., and G. F. Knowlton. 1943. A taxonomic and distributional study of Simuliidae of western United States. Ann. Ent. Soc. America **36**: 259–80.

Stone, Alan, and H. A. Jamnback. 1955. The black flies of New York State (Diptera:Simuliidae). New York State Museum. Bull. **349**: 1–138.

Twinn, C. R. 1936. The blackflies of eastern Canada (Simuliidae, Diptera). Canadian Journ. Res. **14**: 97–150.

Underhill, G. W. 1944. Blackflies found feeding on turkeys in Virginia (*Simulium nigroparvum* Twinn and *Simulium slossonae* Dyar and Shannon). Virginia Agric. Expt. Sta. Tech. Bull. **94**: 1–32.

[3] An aromatic petroleum solvent manufactured by Socony-Vacuum Oil Company, Inc., New York 17, N. Y.

Transmission of T_3 Severe Strain of Tristeza Virus of Citrus by the Melon Aphid[1]

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ABSTRACT

Mild strains of the tristeza virus of citrus have been transmitted experimentally by several species of aphids. Recent tests show that the melon aphid (*Aphis gossypii* Glov.) is also capable of transmitting the severe T_3 strain of this virus. The sources of inoculum were young potted pineapple orange, Cleopatra mandarin, and rough lemon seedlings grown under greenhouse conditions and inoculated with the T_3 strain by means of tissue grafts. These plants were tested for presence of this strain following inoculation by tissue grafts to healthy Key lime indicator plants. Large numbers of these aphids were fed on the source plants for 24 hours, and then were transferred in colonies averaging 300 to Key lime indicator plants for a minimum of 6 hours for transmission feeding. Symptoms characteristic of the T_3 strain of tristeza appeared on 11 of 16 indicator plants in 94 days.

The existence of tristeza virus strains is widely recognized (McClean 1950, Grant & Costa 1951, Hughes & Lister 1953, Norman & Grant 1956, Olson 1956). Tristeza virus has been transmitted by the brown citrus aphid (*Aphis citricidus* (Kirk.)) in several countries (Valiela 1948, Bennett & Costa 1949, McClean 1950, Hughes & Lister 1953). The melon aphid (*Aphis gossypii* Glov.) was not demonstrated to be a vector in limited tests in Brazil (Bennett & Costa 1949), but transmitted the quick-decline or tristeza virus in California (Dickson *et al.* 1956). In Florida mild strains of tristeza virus have been transmitted experimentally by the melon aphid, the spirea aphid (*Aphis spiraecola* Patch), and in one instance by the black citrus aphid (*Toxoptera aurantii* (Fonsc.)) (Norman & Grant 1954, 1957). Although these species were vectors of the mild strain in Florida, they had not been proved to be vectors of the T_3 severe strain described by Grant & Higgins (1957).

In initial vector tests at Orlando, Florida, for transmission of the T_3 virus strain, acquisition feeding was on tissue-inoculated sweet orange, Orlando tangelo, and sour orange grown in a nursery-type planting. No transmission of the virus to Key lime by the aphids was obtained. It was then decided to repeat the transmission attempt, using improved sources of inoculum and increasing the aphid populations. A note on the successful transmission of the strain by the melon aphid has been published (Norman 1958). The present paper describes in more detail the techniques used and discusses their significance in relation to results.

Since the aphid species available for tests were believed

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The identity of the aphid species was confirmed by Louise M. Russell, of the Entomology Research Division.

Table 1.—Aphid transmission of T_3 severe tristeza virus to Key lime indicator plants.[a]

Aphid Species	Number of Aphids per Test	Sweet Orange	Cleopatra Mandarin	Rough Lemon	Date of Transmission (1957)
Spirea aphid	50–200	0/5	0/2	0/4	Jan.–Feb.
Black citrus aphid	50–400	0/32	0/2	0/2	Oct.–Nov.
Melon aphid	200–400	10/12	0/2	1/2	June

[a] Numerator indicates number of plants infected; denominator indicates total number of test plants exposed.

to be inefficient vectors (Dickson *et al.* 1956, Norman & Grant 1954, 1957), every effort was made to establish optimum sources of inoculum and optimum condition of test plants at the time of insect transfers.

MATERIALS AND METHODS.—The sources of inoculum for insect feeding were young potted seedlings (pineapple orange, Cleopatra mandarin, and rough lemon) grown under greenhouse conditions. The pineapple orange seedlings were inoculated with the T_3 severe strain by means of three leaf pieces, as described by Grant & Higgins (1957). The seedlings were cut back to a single 8-inch main stem at the time of inoculation to force new growth. Seedlings of the other varieties were smaller, so that only two leaf pieces were used to inoculate the single main stems cut back to 6 inches in height. Presence of the severe strain of tristeza virus in the new growth of these source plants was confirmed by tissue transfers to Key lime test plants.

Tests for aphid transmission of the virus were made 90 to 112 days after the seedling sources of inoculum had been tissue-inoculated and while the plants provided a growth flush most favorable for aphid feeding. Most of the caged apterous melon aphids were reared on potted healthy hibiscus seedlings, and some were reared on potted healthy citrus seedlings. The apterous spirea aphids and the black citrus aphids were reared on caged Temple orange trees growing in 10-gallon crocks. Infested leaves and shoots were placed on the seedling sources of inoculum and the aphids were allowed to move to the seedlings voluntarily.

After an acquisition-feeding period of 24 hours, the infested leaves and shoots were placed on the Key lime indicator plants until a colony averaging 300 aphids was established on each plant. A minimum transmission-feeding period of 6 hours was allowed. The indicator plants, as a result of prior cutting back, had numerous succulent branches and new leaf growth on which the aphids fed. The container-grown plants were maintained under greenhouse or cage conditions at all times.

RESULTS.—The data in table 1 show that the spirea and the black citrus aphids failed to transmit the severe strain of tristeza virus in these tests. In contrast, the melon aphid[2] infected Key lime plants with the virus showing typical symptoms as shown in figure 1 in comparison with a healthy plant. At the end of 10 months it was noted that 5 of the 11 infected plants exhibited extreme stunting, some vein corking on the upper surfaces of the older leaves, leaf drop, and branch die-back; 5 other plants remained stunted, with leaf cupping and yellowing; and all these 10 plants had very thick bark and showed stem pits and striations. The one remaining plant showed some new growth and a slight tendency toward the production of milder symptoms, but it had thick bark with distinct pits and striations separated by normal-appearing wood. The leaves of plants upon which the spirea and black citrus aphids fed were normal in growth, showed no indications of vein-clearing, and the wood showed no stem pits or striations.

This successful transmission of the T_3 severe strain by apterae suggests that transmissions by alatae could occur

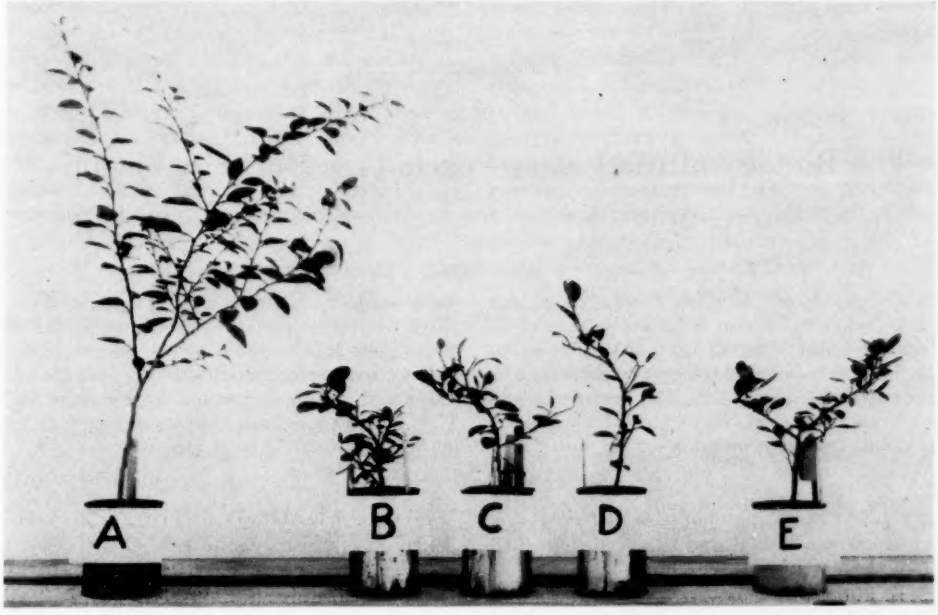

FIG. 1.—Key lime plants 7 months after inoculation with the T_3 severe strain of tristeza virus: A, Control, not inoculated; B, C, D, inoculated by the melon aphid transferred from pineapple sweet orange sources; and E, inoculated by leaf-piece transfer from the standard T_3 source of tristeza virus.

under field conditions. Additional tests will be made to determine if alatae can transmit tristeza. In an earlier experiment using the mild tristeza virus as the source of inoculum and 10 to 50 melon aphid alatae per Key lime indicator plant, no transmissions were obtained in 55 trials. However, according to Severin & Freitag (1938), Costa & Grant (1951), and Sylvester (1954), there is little evidence of a great difference between the ability of alatae, apterae, or nymphs to transmit nonpersistent viruses.

The transmissions provide a means of establishing for experimental purposes T_3 virus sources that can be considered to be free of several citrus viruses not known to be transmitted by aphids. The optimum sources of inoculum and prime condition of the test plants may have been important factors in the high rate of transmission obtained—to 11 of 16 test plants—with the melon aphid. It is also possible that the particular collection of melon aphids from which the larger test colonies were reared represented an especially capable vector strain. Transmission was obtained with both infected rough lemon and sweet orange serving as virus sources. In these tests the melon aphids reared on hibiscus seedlings were as efficient vectors as those reared on young citrus seedlings.

In California the spirea aphid and the black citrus aphid have not been shown to be vectors of the virus. In Florida the spirea aphid has transmitted the common, mild strain of tristeza in a number of tests, but the black citrus aphid has transmitted this strain only once. These differences in ability to transmit T_3 tristeza virus also suggest the existence of aphid strains that vary markedly in vector efficiency.

REFERENCES CITED

Bennett, C. W., and A. S. Costa. 1949. Tristeza disease of citrus. Jour. Agric. Res. **78**(8): 207–37.

Costa, A. S., and T. J. Grant. 1951. Studies on transmission of the tristeza virus by the vector, *Aphis citricidus*. Phytopath. **41**(2): 105–13.

Dickson, R. C., Metta McD. Johnson, R. A. Flock, and Edward F. Laird, Jr. 1956. Flying aphid populations in southern California citrus groves and their relation to the transmission of the tristeza virus. Phytopath. **46**(4): 204–9.

Grant, Theodore J., and A. S. Costa. 1951. A mild strain of the tristeza virus of citrus. Phytopath. **41**(2): 114–22.

Grant, Theodore J., and Richard P. Higgins. 1957. Occurrence of mixtures of tristeza virus strains in citrus. Phytopath. **47**(5): 272–6.

Hughes, W. A., and C. A. Lister. 1953. Lime dieback in the Gold Coast, a virus disease of the lime, *Citrus aurantifolia* (Christmann) Swingle. Jour. Hort. Sci. **28**(2): 131–40.

McClean, A. P. D. 1950. Virus infections of citrus in South Africa. Farming in S. Africa **25**: 261–2, 289–96.

Norman, Paul A., and Theodore J. Grant. 1954. Preliminary studies of aphid transmission of tristeza virus in Florida. Florida State Hort. Soc. Proc. (1953) **66**: 89–92.

Norman, Paul A., and Theodore J. Grant. 1957. Transmission of tristeza virus by aphids in Florida. Florida State Hort. Soc. Proc. (1956) **69**: 38–42.

Norman, Paul A. 1958. Severe T_3 strain of tristeza virus transmitted by *Aphis gossypii* Glover. Jour. Econ. Ent. **51**(1): 45 (Sci. Note).

Olson, Edward O. 1956. Mild and severe strains of tristeza virus in Texas citrus. Phytopath. **46**(6): 336–41.

Severin, Henry H. P., and Julius H. Freitag. 1938. Western celery mosaic. Hilgardia **11**(9): 494–558.

Sylvester, Edward S. 1954. Aphid transmission of nonpersistent plant viruses with special reference to the *Brassica nigra* virus. Hilgardia **23**(3): 53–98.

Valiela, M. V. Fernandez. 1948. Informe preliminar acerca de la etiologia de "podredumbre de las raicillas" del naranjo agrio injertado (Argentina). Rev. Invest. Agric. **2**: 139–46.

The Biology of the Lesser Peach Tree Borer in Virginia[1]

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ABSTRACT

The adult female of the lesser peach tree borer, *Synanthedon pictipes* (G. & R.), prefers to oviposit in broken bark, and no evidence was found to indicate that larvae initiated injury to young trees. The incubation period of the eggs varied from 8 to 20 days, depending upon the season of the year. Large numbers of newly hatched larvae died before becoming established in the trees, and the highest survival occurred when the larvae had ready access to active wounds. Two generations of the lesser peach tree borer develop in Virginia each year. The first brood larvae grow rapidly and complete their development in about 50 days, but the second brood larvae pass the winter in various stages and require approximately 240 days for development. The cocooned prepupal stage lasts from 3 to 7 days, and the pupal period from 15 to 28 days.

The lesser peach tree borer, *Synanthedon pictipes* (G. & R.), is a native American insect, and was first described in 1868. Most of the early records report this species as being injurious to plum and cherry trees, and it was not until Quaintance (1906) had reported it as having caused considerable injury to peach trees in Georgia that it was considered a pest of importance to peach trees. Girault (1907) and King (1917), and later Gilbertson (1934), published on life history studies of this species, but in recent years only brief notes on the biology of the lesser peach tree borer have been published. Smith (1951) stated that there is only one generation a year in New York, but King & Morris (1956) found a high percentage of the

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lesser peach tree borers completing two generations in east Texas.

During recent years the lesser peach tree borer has seriously injured many trees in eastern Virginia and is becoming more numerous in peach orchards throughout the State. This report covers biological studies and observations during the years 1956 through 1958.

METHODS.—To determine the development of the immature stages during the season, larvae and pupae were removed from peach trees at 2-week intervals from March through November, and at longer time intervals throughout the remainder of the year. The width of the head capsules of the larvae was measured. Adult emergence was determined by observations at 2-week intervals of empty pupal skins from five marked trees. All cast skins were destroyed after examination.

The pupae collected were used to rear moths for egg deposition and subsequent incubation data. The moths were confined in glass battery jars, the tops covered with cheesecloth, and the jars turned on their sides. The moths readily oviposited through the cloth.

Field observations of moth activity and habits were made at all times of the day, and individual females were observed for long periods to determine their oviposition characteristics. Pieces of bark containing eggs deposited by the moths under observation were collected. The eggs, as well as the head capsules of the larvae, were measured with a micrometer eyepiece in a binocular microscope.

RESULTS.—The male and female moths of the lesser peach tree borer resemble each other very closely in color, size, and markings, and both sexes have transparent wings. The male may be distinguished from the female, however, by the finely tufted antennae and the more slender body.

The adult female prefers to oviposit in broken bark and, except under conditions of extremely heavy infestations, deposits very few eggs on young trees which have smooth bark with no injuries. The presence of external gum, mixed with frass from larval feeding, has a marked influence on the female when selecting a location on which to deposit her eggs. This condition is most often found in vigorous trees, and Smith & Harris (1952) have shown a positive correlation between tree vigor and borer infestation. They state that a more favorable condition for oviposition and subsequent larval establishment is afforded by wounds on trees high in vigor.

When ovipositing, the female flies up and down the limbs or tree trunk until a suitable place for ovipositing is found. She then alights and walks over the surface probing her ovipositor into cracks and crevices and under bark scales. When a suitable location for the egg is found, she pauses, thrusts the ovipositor deeper, and with wavering movements of the abdomen deposists an egg. She then usually takes to flight but may return to the same location to deposit another egg. Although a female will usually deposit only one egg at a time, as many as 54 eggs have been found under a single small piece of bark. This may be attributed to the same female, as well as to others, ovipositing more than once in a given location. Large numbers of eggs are deposited in and around active wounds. After the female deposits from four to six eggs, she seemingly becomes exhausted and alights on a tree limb to rest. At this time the moth may be easily captured by merely picking her up with the fingers, since she has no inclination to fly, although they are normally very swift and active fliers.

Moths reared from pupae confined in battery-jar cages in the insectary deposited large numbers of eggs, but all were infertile and did not hatch. Field-collected adult females, however, deposited fertile eggs in captivity.

The eggs are small, averaging 0.4×0.6 mm., reddish-brown in color, and extremely hard. The chorion retains its shape after the egg has hatched and the only evidence of hatching is the small exit hole of the larva at the anterior end. The incubation period of eggs kept in the insectary varied from 17 to 20 days during May to 8 to 12 days during midsummer. Observations in the orchards, however, indicate that there is a period of approximately 25 to 30 days from peak moth emergence to peak egg hatching during the first brood, and approximately a week or 10 days during the second generation.

The larvae when first hatched are barely visible to the naked eye and are difficult to detect in the bark of trees, but after a few days a small pile of reddish wood dust marks their presence. They are partial to wounds and become established most often in split limbs and crotches, in pruning scars, in abrasions caused by equipment, and in winter-injured areas of the bark. No evidence was found to indicate that larvae initiated injury in young trees, and attempts to establish them in such trees have been in vain. However, heavy infestations have become established during a single season in old uninjured trees with rough bark.

Large numbers of the newly-hatched larvae die before becoming established in the tree and the highest survival occurred when the larvae had ready access to active wounds. After becoming established in an area on a tree the larvae feed on the tender bark around the margins of the wound. They rapidly increase the wound area and feed on the cambium and inner bark layers. The larvae have a voracious appetite, and the feeding of several hundred borers in a tree may completely honeycomb large limbs and cause them to die; or entire trees have been observed killed when high borer infestations occurred in the trunk. The larvae do not have cannibalistic tendencies, which fact permits large numbers of them to mature in a small wound on a tree. From one such wound, about 4 by 7 inches in size, 62 first brood moths and 49 second generation moths emerged during 1956; and from another wound which had been made on the trunk of a 3-year-old tree by a disk while cultivating 2 years previously, 92 moths emerged between May 2 and July 25. Infestations of the lesser peach tree borer occur most often on the trunk and larger limbs of peach trees, but many larvae are sometimes found in wounds on smaller limbs in the tops of trees, and a few borers may even be found below the ground level in association with the peach tree borer.

It is difficult to observe the larvae at regular intervals, and there is no direct evidence to show how many instars there are, but Girault (1907) has stated that there are six larval instars. Head capsule measurements of 842 larvae varied greatly, and the frequency distribution indicated that 5, 6, or 7 instars are required for larval development. The majority of the larvae probably pass through six instars, and table 1 gives the range- and average head-

Table 1.—Head capsule measurements of lesser peach tree borer larvae collected from peach trees at Hampton, Virginia, during the years 1956 to 1958, inclusive.

Larval Instar	Number of Individuals	Width of Head Capsule (mm.)	
		Range	Average
First	25	0.23–0.35	0.28
Second	79	0.41–0.64	0.53
Third	91	0.67–0.99	0.83
Fourth	157	1.02–1.34	1.18
Fifth	181	1.37–1.81	1.62
Sixth	282	1.84–2.39	2.06
Cocooned larvae	27	1.96–2.34	2.07

capsule measurements for the six instars.

The first brood larvae grow rapidly and complete their development in about 50 days, but the second brood larvae pass the winter and therefore require approximately 240 days for completion of development. The winter is passed in various stages of larval development under the bark and in old feeding galleries. All instars from the second through the sixth have been collected during the winter months (fig. 1). The majority of the overwintering larvae resume feeding in late winter and early spring and, as they reach maturity, construct cocoons and pupate. Observations indicate, however, that a few larvae complete their feeding in the fall and pupate with the approach of warm weather without further feeding.

After larval development has been completed, the larva constructs a cocoon just under the bark or near the surface of the gum and frass surrounding the wound. The larva becomes thicker and shorter, and pupates in from 3 to 7 days. From 15 to 28 days are spent in the pupal stage, depending upon the season of the year, after which time the pupa pushes its way through the cocoon and out through the bark until one-half or more of it projects from the tree. The moth then emerges, leaving the old pupal case projecting from the bark.

Two generations of the lesser peach tree borer develop in Virginia each year. The first generation is passed in approximately 3 months, but the second generation requires 9 months, as the larvae do not complete their development and the moths do not emerge until the following spring. Under normal orchard conditions the second generation is the larger one; this may be attributed to early sprays, especially parathion, which reduces the numbers of the first generation.

Spring brood moths begin to emerge in early April and they continue to emerge until late July. First brood moths emerge from early July through November, there being an overlapping of the generations during July. Adults which emerge during late October and November have never been observed to deposit eggs.

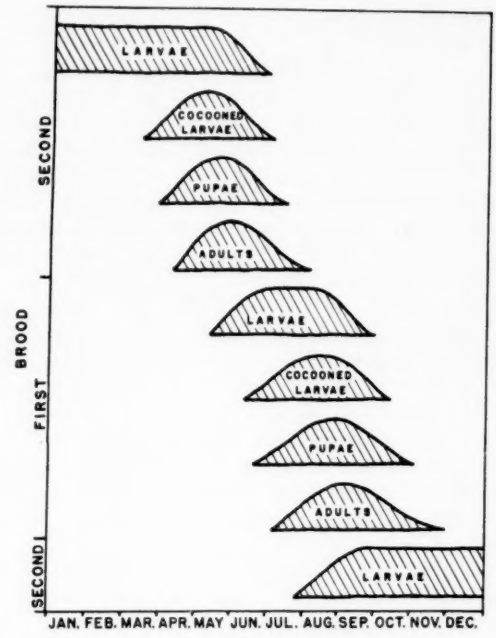

FIG. 1.—Seasonal history of the larval, pupal and adult stages of the lesser peach tree borer as indicated by sampling. The curve for each stage of development shows the beginning, ending, and peak.

REFERENCES CITED

Gilbertson, G. I. 1934. The plum tree borer, its distribution, life history, economic importance and control. South Dakota Agric. Expt. Sta. Bull. **288.**

Girault, A. A. 1907. The lesser peach tree borer. U. S. Dept. Agric. Bur. Ent. Bull. **68.**

King, J. L. 1917. The lesser peach tree borer. Ohio Agric. Expt. Sta. Bull. **307.**

King, D. R., and H. F. Morris. 1956. Biologies of the peach tree borer and lesser peach tree borer in east Texas. Jour. Econ. Ent. **49**(3): 397.

Quaintance, A. L. 1906. The principal insect enemies of the peach. U. S. Dept. Agric. Yearbook for 1905, 325–48.

Smith, E. H. 1951. Control of peach tree borer and lesser peach tree borer in New York. Jour. Econ. Ent. **44**(5): 685–90.

Smith, E. H., and R. W. Harris. 1952. Influence of tree vigor and winter injury on the lesser peach borer. Jour. Econ. Ent. **45**(4): 607–10.

Control of Peach Twig Borer on Almonds and Peaches in California[1]

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ABSTRACT

Numerous recently introduced and staple insecticides were tested for effectiveness against the peach twig borer, *Anarsia lineatella* Zell. Initial or preliminary evaluations were obtained by spraying small, nonbearing peach or almond trees and counting soon thereafter the twig terminals damaged by surviving larvae. The experimental insecticides which gave promising results in more than one of these twig-strike tests were further tested on mature peach trees for appraisal based on reduction of crop damage. Data obtained from all these tests indicate that, within the range of periods and dosages tried, Sevin® (1-naphthyl-*N*-methyl carbamate) and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) have good potentialities for use against this pest of stone fruits. Sevin 50 WP and Thiodan 25 WP tended to yield consistently lower twig borer counts in twigs and fruits than DDT 50 WP—each used at 2 pounds of formulated material per 100 gallons—but the differences observed were not significant in every trial. Other insecticides which yielded low-strike counts in one or more of the trials on nonbearing trees were Guthion® (*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate), Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), dieldrin, endrin, and malathion. Two applications of each of six insecticides tested on mature peach trees, one application in the pink bud or petal fall period and a second application for the May generation of larvae, reduced crop infestation to reasonably low levels but not within the range of safe or assured commercial control under conditions of severe infestation.

The peach twig borer, *Anarsia lineatella* Zell., is a chronic pest of peach orchards in the central valleys of California. During the past 4 years its depredations have increased so markedly that normally successful control procedures no longer suffice for orchards in several seriously infested areas. It is desirable, therefore, to reappraise the performance of standard chemicals and to identify optional chemicals which may be well adapted to this use. This report presents results from two seasons of test work in which new or experimental chemicals were compared with currently used materials such as DDT, parathion, and lead arsenate.

TESTS ON NON-BEARING TREES.—It is frequently possible to obtain a tentative rating of test materials by spraying young almond or peach trees before a generation of larvae begins to infest the foliage, by recording results at the close of the feeding period in terms of numbers of twigs mined by survivors of the generation treated (Summers 1951). The over-wintered generation of larvae (which invades the foliage during the blooming period of the host trees) and the first spring generation (*i.e.*, "May brood" which appears in mid-May) can be utilized satisfactorily for this type of testing since the larvae of these generations are predominantly twig feeders.

The first experiment of this category included 11 test materials applied to heavily infested young peach trees (table 1). All the sprays listed in this series reduced the worm population appreciably. Those containing endrin, Sevin® (1-napthyl-*N*-methyl carbamate), parathion, Guthion®(*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate), and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) appeared to be significantly more effective than all the others except the spray of Trithion® (*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate). The first five materials listed in table 1 reduced the numbers of survivors to such low levels that the observer could identify little or no evidence of injury to terminal shoots on the sampling date. The plots treated with toxaphene or DDT were indistinguishable from each other and both fell in the middle range of values recorded for the experiment.

Available criteria for estimating levels of orchard infestation are often not sufficiently reliable to assure success in trials of this type. The orchard in which the first experiment was completed provided an unusually favorable situation for another round of tests against the next generation of larvae. It was not commercially sprayed in the petal fall period for twig borer. Accordingly, another series of test sprays was applied elsewhere in the same orchard during May.

Results obtained in the second series of tests are given in table 2. In this series, as in the first, Thiodan, endrin and Sevin reduced the numbers of strikes to zero, or nearly so. Not assuredly distinguishable from each other in a middle range group were plots treated with toxaphene plus DDT, DDT (3 lbs. 50 WP) and Trithion. While it was not advisable to leave an untreated check plot in this test, the numbers of strikes appearing in the remainder of the orchard after the owner treated with DDT revealed that the infestation carried over at a high level.

Three series of peach plots treated in the petal fall period of the next season, 1958, did not have populations adequate for distinguishing between treatments. However, another twig-strike test applied in 1958 to almond trees for larvae of the May brood yielded interpretable information (table 3). Almonds are cross-pollinated and orchards are therefore usually planted with at least three varieties. Good plot management in this test orchard required that the test sprays be divided between two varieties because it was anticipated that only these varieties would continue their succulent growth through the larval feeding period and there were not enough rows of either variety to fill the requirements of the test.

Trees of the Texas variety sprayed with dieldrin, malathion, and Thiodan had fewer strikes than those sprayed with Dibrom® (*O,O*-dimethyl-*O*-(1,2,-dibromo-2,2-dichloroethyl) phosphate) and ethion at dosages indicated (table 3). Similarly, on trees of the NePlus variety, those sprayed with Sevin, Diazinon, and DDT showed fewer strikes than those sprayed with Perthane® (1,1-dichloro-2,2-bis(*p*-ethylphenyl ethane) and Trithion. Trees

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Table 1.—Effect of petal fall sprays on the reduction of twig borer larvae of the overwintered generation on nonbearing Vivian cling peach trees. Modesto, California, 1957.[a]

MATERIALS	POUNDS PER 100 GALLONS	TOTAL NUMBER OF STRIKES ON SIX TREES[b]
Endrin 1.6 EC	1 pt.	0
Sevin 50 WP	2	0
Parathion 25 WP	2	0
Guthion 25 WP	2	2
Thiodan 25 WP	2	5
Trithion 25 WP	2	14
Toxaphene 40 WP	2	32
DDT 50 WP	2	38
Navadel 25 WP	2	49
Lead arsenate, basic	4	55
Ethion 25 WP	2	77
Check	—	571
L.S.D. at 5% level		22

[a] Third-leaf trees sprayed March 21 (petal fall), 40 to 45 gallons per 20 trees per plot, drenching coverage at 400 p.s.i., by hand.

[b] Counts of strikes made on April 26; sampling restricted to 6 trees per plot, each 6 in corresponding locations within all plots.

sprayed with endrin were not as worm free as those treated with Sevin but they were at least as disinfested as those treated with Diazinon and DDT.

TESTS ON FULL-BEARING TREES FOR HARVEST DATA.—Three of the experimental insecticides identified as potentially good in the twig-strike tests—Sevin, Thiodan, and Trithion—were selected for further trial on peaches for evaluation in terms of reduction of crop damage. Three insecticides currently used for twig borer control were included for comparison—DDT, parathion, and basic lead arsenate.

Three groups of plots, 100 Fortuna trees per plot, were sprayed so that each material was applied at periods appropriate for first or first and second generation worms, *viz.*, 1) pink bud only, 2) pink bud and May, 3) petal fall and May. Each group comprised 9 plots arranged in a 3×3 pattern, with the center plot left as an untreated check. Unused plots in the first two groups were treated as spare DDT plots. All the applications were made with a blower sprayer, with dates and average volumes for 90 trees per acre as follows: pink bud, March 3 to 5, 400 gallons; petal fall, March 26, 500 gallons; May 20, 600 gallons. Each material was used at the same dosage in each application. The dosages of formulated materials used per 100 gallons were as follows: Thiodan 25 wettable powder (WP)-2 pounds, Sevin 50 WP-2 pounds, basic lead arsenate-4 pounds, DDT 50 WP-2 pounds, parathion 25 WP-2 pounds, and Trithion 4 Flowable-1 pint. The May applications also included parathion emulsion concentrate (EC) (Orthophos 4 Spray, California Spray-Chemical Corp.) at ½ pint and 1 pint dosages. The entire planting appeared to be heavily infested initially and the three check plots were critically but not equally infested during the harvest period.

The counts of wormy fruits obtained from plots treated during each of the growing periods are entered in table 4 as percentages based on ten 100-fruit samples. All peaches removed from the trees by pickers were retained for examination.

The amounts of worm-damaged fruit taken from plots sprayed with basic lead arsenate and with DDT were unexpectedly low in these limited commercial trials. Only lead arsenate yielded averages consistently in the top bracket for the three groups of applications. Numbers of wormy peaches picked from plots sprayed with parathion WP were not significantly lower than those taken from plots sprayed with DDT; in fact, the counts tended to favor DDT over parathion WP in the three groups. Plots treated twice with Thiodan, lead arsenate, and Sevin, at dosages used, were not clearly distinguishable from each other. Plots treated with two dosages of parathion EC, 0.25 and 0.5 pounds actual, in the petal fall-May series were not distinguishable from each other, but both of these yielded significantly fewer infested fruits than the

Table 2.—Trials of several pesticides on nonbearing Vivian cling peach trees to control peach twig borer larvae of the May generation. Modesto, California, 1957.[a]

MATERIALS	POUNDS PER 100 GALLONS	TOTAL NUMBER OF STRIKES ON 10 TREES[b]
Thiodan 25 WP	2	0
Endrin 1.6 EC	1 pt.	1
Sevin 50 WP	2	3
Toxaphene 40 WP+DDT 50 WP	2+1	22
DDT 50 WP	3	38
Trithion 25 WP	2	47
DDT 50 WP[c]	2	111
L.S.D. at 5% level		37

[a] Third-leaf trees sprayed May 15, at the onset of hatching of eggs of the first generation (i.e., "May Brood"). Twenty trees sprayed per plot with drenching coverage, by hand.

[b] Counts of strikes made on June 3, as larvae of this generation passed the peak of maturity. Ten trees used for counting were selected only for uniformity of size.

[c] Owner's, May 26.

Table 3.—Trials of various pesticides on 4-year almond trees to control peach twig borer larvae of the May brood. Arbuckle, California, 1958.

MATERIALS	AMOUNTS PER 100 GALLONS	TOTAL NUMBERS OF STRIKES ON SIX TREES PER PLOT[1]
Texas Variety		
Dieldrin 50 WP	1 pound	3[a]
Malathion 25 WP	3 pounds	4[a]
Thiodan 2 Misc.	1 quart	7[a]
Dibrom 8 EC	1 pint	20[b]
Ethion 4 Misc.	1 pint	60[a]
Check	—	201
Check	—	242
Check	—	246
NePlus Variety		
Sevin 50 WP	2 pounds	0[a]
Diazinon 25 WP	2 pounds	4[ab]
DDT 50 WP	2 pounds	4[ab]
Endrin 1.6 EC	1 pint	14[bc]
Perthane 2 EC	2 quarts	31[cd]
Trithion 4 Flow.	0.5 pint	62[d]
Check	—	174
Check	—	200
Check	—	300

[1] Statistical analysis performed on transformed data, using the transformation $\sqrt{X+0.5}$. Checks were not included in the analysis. Totals associated with the same superscript letter are not significantly different at the 5% level, based on Duncan's Multiple Range Test (LeClerg 1957).

Table 4.—Effectiveness of various insecticides used for controlling peach twig borer in Fortuna cling peaches. Merced, California, 1958.[1]

Number of Times Sprayed					
Once		Twice		Twice	
Mar. 3 Pink Bud		Mar. 3 and May 20 Pink Bud and May		Mar. 26 and May 20 Petal Fall and May	
Materials	Mean Per Cent Wormy Fruit[2]	Materials	Mean Per Cent Wormy Fruit[2]	Materials	Mean Per Cent Wormy Fruit[2]
Lead Arsenate	20.1[a]	Thiodan	0.8[a]	Thiodan	1.3[a]
Trithion	31.7[ab]	Lead Arsenate	1.3[ab]	Lead Arsenate	1.3[a]
Thiodan	37.9[bc]	Sevin	2.1[ab]	Parathion EC (1 pt.)	1.4[a]
DDT	41.3[bc]	DDT	4.4[ab]	Parathion EC ($\frac{1}{2}$ pt.)	2.1[ab]
Parathion WP	46.3[c]	Parathion WP	5.2[b]	Sevin	3.2[abc]
Sevin	47.8[c]	Trithion	10.1[c]	DDT	8.3[bc]
—	—	—	—	Parathion WP	8.6[c]
—	—	—	—	Trithion	22.5[d]
Check	69.4	Check	36.7	Check	32.0

[1] Ripe peaches harvested and classified on July 9–10.
[2] Means associated with the same superscript letter are not significantly different from each other at the 5% level. Checks not included in the analyses.

plots sprayed with parathion WP at 0.5 pound actual per 100 gallons. Applications of Trithion 4 Flowable to first and second broods of larvae yielded comparatively large numbers of worm-infested peaches. Its higher rank in the pink bud series was not consistent with its performance in the two-spray series.

All the plots sprayed only once during the pink bud period showed perceptible improvement over the corresponding check plot but even the best of the one-spray treatments did not give adequate control against an infestation of this intensity.

The repetition of each spray during May after applications at either of the earlier periods yielded lower average amounts of infested fruit than the corresponding sprays applied once in the pink bud period. Despite minor fluctuations in the ranking of individual treatments within each of the two-spray series, the averages recorded for each of the sprays tend to favor one combination (pink bud and May) over the other (petal fall and May).

DISCUSSION AND INTERPRETATION OF RESULTS.—Final ratings of insecticides for effectiveness against twig borer require a backlog of data from both test plots and commercial experience. Ratings of materials in the good or excellent categories are very difficult to fix with certainty in virtue of local variations in population density and possibly changes in susceptibility.

It is feasible to utilize the "twig-strike" procedure as an aid to selection and rating. The procedure is regarded as a quick "roughing-out" technique which can be completed within the space of one generation of larvae. But its success hinges upon the experience of the operator in appreciating favorable conditions of density and distribution of the pest, growth characteristics of the host trees, etc. Effective materials which reduce numbers of strikes to zero cannot be further differentiated with this procedure.

Data from the four experiments described indicate that, within the range of periods and dosages tested, Sevin and Thiodan have good potentialities for development as new front-line insecticides for controlling peach twig borer. Others which showed promise in one or more of the preliminary tests are Guthion, Diazinon, dieldrin, endrin, and malathion. Materials which appeared to be less effective for twig borer than those named above are ethion, Trithion, Dibrom, Navadel® (2,3-*p*-dioxanedithiol *S*,*S*-bis(*O*,*O*-diethyl phosphorodithioate)), Toxaphene, and Perthane.

The relative effectiveness of DDT and lead arsenate for twig borer control possibly varies according to locality, as these data appear to show. The results given in table 1, for Modesto, accord with local experience. About 10 years ago lead arsenate appeared to fail in this locality (Summers 1949) even as DDT is now appearing to fail. These growers now obtain satisfactory control with parathion; they question the effectiveness of DDT and are reluctant to trust lead arsenate. The yield data obtained in the Merced district, about 36 miles south of Modesto, gave an inverted order of rating, with lead arsenate over DDT and parathion (table 4). The apparent superiority of lead arsenate in this experiment is difficult to accept unless confirmed by more exhaustive study.

Application of each test material twice—once to larvae of the overwintered generation during either pink bud or petal fall periods and again when eggs of the May generation began to hatch—reduced crop infestation to reasonably low levels. However, the best four or five treatments listed in table 4 do not fall within the range of safe commercial control. These data point up a generalization that certainly one and probably two applications of the insecticides known to be effective against twig borer do not assure adequate control against very heavy infestations of this pest.

REFERENCES CITED

Summers, F. M. 1949. Resistance to basic lead arsenate by the peach twig borer within a small area in central California. Jour. Econ. Ent. 42(1): 22–4.

Summers, F. M. 1951. Tests of new materials to control peach twig borer on almonds and peaches. Jour. Econ. Ent. 44(6): 935–9.

LeClerg, E. L. 1957. Mean separation by the functional analysis of variance and multiple comparison. ARS-20-3, U. S Dept. Agric., May 1957.

Observations on Commercial Application of Insecticides for Control of Sap Beetles on Sweet Corn[1]

J. T. Whitlaw, W. G. Phillips, and L. P. Ditman

Abstract

Seven fields of canning sweet corn ranging from 3.0 to 40 acres were treated by airplane with phosphate insecticides in oil. Two fields received 1 pint of parathion emulsifiable in 1 gallon of oil per acre, 1 field 1 pint of parathion in 2 gallons of oil, 2 fields were treated with 1 quart of Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methol-6-pyrimidinyl) phosphorothioate) in 2 gallons of oil and 2 fields received 1 quart of malathion in 2 gallons of oil per acre. All insecticides reduced the initial sap beetle populations. The greatest reduction and the lowest rate of reinfestations occurred in malathion-treated fields. The percentage of ears free of sap beetle at harvest time was reasonably well associated with reduction of the initial infestation. Residues of parathion and Diazinon were more persistent than malathion residues when applied in oil. In these results higher reduction of sap beetle infestations was associated with the higher rate of oil used. It was evident that treatment should be made when the adult population is highest before invasion of the ears has taken place. Observations indicated that this occurred when about 50% of the plants were in silk, although the uniformity of plant development, the susceptibility of varieties (NK199, Illinois 13, and Iowa Chief are considered very susceptible to sap beetle invasion) and the seasonal fluctuation in sap beetle populations should influence the most favorable time of treatment.

The sap beetles, primarily *Carpophilus lugubris* Murr. in Maryland and Delaware (Connell 1956), are serious pests of sweet corn, causing direct injury which results in increased cost of processing and creating a serious contamination hazard in the frozen and canned products. In Maryland sap beetle populations in canning sweet corn reach a peak during July and early August and then gradually decline until by the latter part of September they are of little importance (Harrison & Ditman 1958). It is believed that the severe infestations of recent years are associated with the increasing use of the mechanical corn picker, which leaves on the ground ears of field corn on which sap beetles reproduce in early spring and late fall (Connell 1956).

Darsie & Connell (1952) reported encouraging results on the control of sap beetles from the use of several phosphate insecticides. Luckman (1958) reported successful commercial results from the use of parathion in oil applied by airplane. As a result of grower interest seven fields of canning sweet corn on the Eastern Shore of Maryland were treated by plane, three with parathion, two with malathion, and two with Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methol-6-pyrimidinyl) phosphorothioate). All insecticides were applied in mineral oil. The concentration of parathion and Diazinon was 25%, while the malathion was 50% emulsifible concentrate.

The most important aims of this work were to secure information on (1) insecticide performance from the standpoint of effectiveness, safety of application, and freedom from hazardous residues; (2) suitability of several oils and minimum effective amount; (3) the most effective time for treatment in relation to the development of the corn plant and the degree of sap beetle infestation; and (4) the general practicability of commercial sap beetle control.

Procedure.—In order to determine the original intensity of infestation and the reduction of populations from insecticide treatments, counts of adult sap beetle were made just prior to treatment and at intervals afterwards over periods of from 11 to 16 days. In estimating populations, the beetles were counted on eight 10-plant samples of corn, one sample in each corner of the field and four samples in a line diagonally across the middle of the field. The corn plants were classified as "not infested," "infested with 1 to 5," "5 to 10" or "more than 10" adult beetles. For simplification in reporting the results here, only the percentage of total plants infested will be used. Samples for residue analysis, which consisted of the third leaf from the top of the plants, were collected at each location where the population counts were made. Leaf samples were always in excess of 250 grams. These samples were placed in plastic bags and brought to the laboratory where they were stripped with appropriate solvent by tumbling in 1-gallon cans. Samples were stripped within 1 day after collections were made and the strippings stored at 0° F. in brown glass bottles until analyzed.

Diazinon residues were determined by the phosphate procedure adapted from the method by Holman (1943). Malathion samples were analyzed for residue by the colorimetric method of Norris *et al.* (1954) and residues of parathion were determined using the colorimetric method described by Averel & Norris (1948). Untreated check samples taken before spraying were analyzed, but the values obtained were not deducted from those found on the treated plants. This seemed practical since Food and Drug Administration officials would not have blanks available when making their analysis.

The insecticides used in these experiments, parathion, Diazinon, and malathion, were applied in either Blandol, obtained from L. Sonneborn Sons, N. F. mineral oil light, manufacturer unknown, or Ortho D soluble, obtained from the California Spray Chemical Company. The Blandol has a viscosity of about 140 whereas the Ortho D soluble has a viscosity of 87 to 89 Saybolt seconds. All have an insulphonatable residue of 96% or above. All treatments were applied in the late afternoon.

The percentage of plants in silk was recorded at the time the pretreatment insect counts were made, and sap beetle infestation of 100 to 200 ears was made on random samples from loads at the processing plant at harvesttime. The nature of the treatments and results are given in table 1.

[1] Scientific Article Number A744, Contribution Number 2992, of the Maryland Agricultural Experiment Station, Department of Entomology. Accepted for publication January 22, 1959.

Table 1.—Results of insecticide treatments applied to commercial plantings of sweet corn to reduce sap beetle infestation. Eastern Shore of Maryland, 1958.

Date Treated and Variety	Days After Treatment	No. of Plants: Examined	No. of Plants: Infested (%)	No. of Plants: Reduction of Infested Plants (%)	Residues on Leaves (p.p.m.)	Silking When Treated (%)	Harvested Ears Free of Sap Beetle (%)
		Parathion 25% E.C. 1 pint in 1 gallon of Blandol (3 acres)					
	0	25	100	—	—	90	
	1	25	81	19	—		
	2	80	59	41	1.60		
July 14	11	80	53	47	.34		
NK199	14	80	50	50	.34		
		Parathion 25% E.C. 1 pint in 1 gallon of Blandol (12 acres)					
	0	50	68	—	—	75	54
	1	50	72	—	—		
	2	80	52	18	3.73		
	8	80	27	60	.48		
	10	80	30	56	1.06		
July 14	14	80	37	46	.94		
NK199	16	80	44	35	.07		
		Parathion 25% E.C. 1 pint in 2 gallons of Ortho D soluble (40 acres)					
	0	80	25	—	—	45	98
	2	80	10	60	6.04		
	6	80	10	60	1.54		
July 19	9	80	14	44	.65		
Asgrow 60	11	80	19	64	.56		
		Diazinon 25% E.C. 1 quart in 2 gallons of Ortho D soluble (20 acres)					
	0	80	91	—	—	45	66
	2	80	18	80	1.80		
	6	80	22	75	.23		
July 19	9	80	24	74	.25		
Iowa Chief	11	80	47	48	2.56		
		Diazinon 25% E.C. 1 quart in 1 gallon N.F. mineral oil light (4.5 acres)					
	0	80	74	—	—	60	43
	3	80	25	66	0.95		
	5	80	18	75	.18		
	7	80	15	80	1.57		
July 25	11	80	29	61	.00		
NK199	14	80	36	51	.00		
		Malathion 50% E.C. 1 quart in 2 gallons Ortho D soluble (8 acres)					
	0	80	68	—	—	55	88
	3	80	5	94	0.58		
	9	80	4	95	.00		
August 2	11	80	5	94	.00		
Illinois 13	13	80	8	88	.00		
		Malathion 25% E.C. 1 quart in 2 gallons Ortho D soluble (12 acres)					
	0	80	27	—	—	55	88
	3	80	5	81	0.68		
	9	80	0	100	.00		
August 2	11	80	4	85	.00		
Illinois 13	13	80	4	85	.00		

DISCUSSION AND SUMMARY.—In these experiments all the insecticides reduced the initial sap beetle populations, the highest reduction being obtained with malathion at 1 quart in 2 gallons of oil per acre and the rate of reinfestation was lowest with the malathion treatment. The percentage of ears free of sap beetles seems to be reasonably well associated with reduction of the initial infestation.

Residues of parathion and Diazinon were more persistent than those of malathion. It is quite probable that no further work will be done with parathion, not only because of the persistence of residue, but also because of its high mammalian toxicity which is of great concern because of the proximity of corn fields to dwellings and pastures. Malathion appears favorable from the standpoint of nonhazardous residues, though early season application may not be as effective as the midseason treatments here reported.

The kind of oil used is probably of little importance to effectiveness of treatment provided it does not injure the corn plant. In these results higher reduction of sap beetle populations is associated with the higher rate of oil used.

It appears self evident that the time for treatment should be when the adult beetle population is highest before invasion of the ears has taken place. Observations indicate that this occurs when about 50% of the plants are in silk, though the uniformity of plant development, the susceptibility of varieties, and the seasonal fluctuation in sap beetle population should be expected to influence the time most favorable for treatment, but considerable work is necessary before all this information can be obtained.

Though observations reported here are not extensive, results are sufficiently encouraging to justify further work.

REFERENCES CITED

Averel, P. R., and M. V. Norris. 1948. Estimation of small amounts of *O,O*-diethyl-*O*-*p*-nitrophenyl thiophosphate. Analyt. Chem. **20**: 753–6.

Connell, W. A. 1956. Nitidulidae of Delaware. Univ. of Delaware Agric. Expt. Sta. Bull. **318** (Tech.) 67 pp.

Darsie, R. F., and W. A. Connell. 1952. Preliminary tests for control of sap beetles in sweet corn. Trans. Penin. Hort. Soc. **42**(5): 18–21.

Harrison, F. P., and L. P. Ditman. 1958. Ecological studies of insects attacking canning sweet corn in relation to timing chemical control. Maryland Agric. Expt. Sta. Tech. Bull. **A-96**. 19 pp.

Holman, W. I. M. 1943. A new technique for the determination of phosphorus by the molybdenum blue method. Jour. Biochem. **87**: 256–9.

Luckman, William H. 1958. Information by correspondence.

Norris, M. V., W. A. Vail, and P. R. Averel. 1954. Colorimetric estimation of malathion residues. Jour. Agric. Food Chem. **2**: 570–3.

Effects of Heavy Fall Infestations of Spotted Alfalfa Aphids[1] on Subsequent Spring Growth of Alfalfa in Kansas[2]

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ABSTRACT

Alfalfa infested with as many as 325 spotted alfalfa aphids (*Therioaphis maculata* (Buckton)) per sweep in the fall after the last cutting had been removed was treated with malathion and BHC at 1 lb. and ¾ lb. gamma isomer per acre, respectively. Excellent control resulted. Analysis of alfalfa pellets from the first cutting the following spring showed protein 2% higher and carotene 3 mg. per 100 grams higher in the treated plot. No increase in yield resulted from the fall treatment.

The spotted alfalfa aphid has been known to affect both the yield and quality of alfalfa hay (Parker *et al.* 1956, Sorensen *et al.* 1958). This insect pest cost Kansas alfalfa growers an estimated 2.4 and 8.5 million dollars during 1955 and 1956, respectively. Heavy infestations often retard or prevent regrowth following cutting.

Many farmers inquire about the practical value of killing the aphids when they exist in large numbers on an established stand of alfalfa in the fall after the last cutting has been removed. It was not known whether the toxic materials injected by the aphids in the fall, or if other injury would increase winter mortality of alfalfa plants in the latitude of Kansas, or whether such a fall attack would affect growth or quality of hay the following spring. This study was conducted to determine what value, if any, could be obtained from controlling heavy fall populations of aphids.

MATERIALS AND METHODS.—A 40-acre, infested field of alfalfa was located north of Manhattan October 9, 1956. The field was harvested by October 12. October 26 the field was again examined. An irrigated field with an adequate water supply, it then had made about 1.5 inches of regrowth. The plants were covered with honey dew and their leaves were turning yellow. Aphid counts averaged 250 per sweep with an insect net.

Plots were established October 26. The 40-acre field was divided into thirds. Plot A was treated with BHC at ¾ pound of gamma isomer per acre. Malathion was applied at 1 pound toxicant per acre to Plot B. Plot C was left untreated as the control. All insecticides were applied with a boom-type sprayer mounted on a jeep. The pump was operated at 30 pounds of pressure, applying 17 gallons of emulsion per acre. Aphid populations were examined following treatments until the time of a killing frost November 14. The following spring, May 1957, the first cutting of alfalfa was harvested. Alfalfa was harvested with field cutters and taken to a dehydrating plant. Yield was based on 3 swaths, each 8 feet wide and 40 rods long, and consisted of almost a truckload. Each load was processed separately with a waiting interval between loads, and yield was recorded in pounds of dry weight. Five random 1-pound samples of alfalfa pellets from each treatment were taken from the flowing stream of pellets immediately following processing and were then analyzed for crude protein and carotene.

RESULTS.—Posttreatment counts showed the number of aphids was significantly reduced in treated plots to <3 aphids per sweep. BHC gave excellent control while malathion gave fair control of the aphids until November 14, when a killing frost occurred. Throughout the posttreatment period of 3 weeks preceding the frost, aphid populations remained heavy and even increased under ideal climatic conditions in the untreated control plots to >325 per sweep.

Yield data for the May 1958 harvest are shown in table 1. These data indicated no substantial differences in yield between treatments.

Results of analysis of crude protein and carotene of alfalfa pellets taken from each treatment are shown in

Table 1.—Yields of first cutting in alfalfa plots, Manhattan, Kansas. May 1957, following spraying during the previous fall.

TREATMENT	POUNDS ACTUAL TOXICANT PER ACRE	POUNDS ALFALFA (DRY WEIGHT)
BHC	0.375 (gamma)	1,595
Malathion	1	1,550
Check	—	1,550

[1] *Therioaphis maculata* (Buckton).

[2] Contribution No. 717, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan. Studies were supported in part by Hatch Project 409. Accepted for publication January 21, 1959.

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Table 2.—Analysis of dehydrated alfalfa pellets from first cutting (table 1).

Treatment	Moisture (%)	Crude Protein (N×6.25)[a]		Carotene (mg./100 gm.)	
		A[b]	B[c]	A[b]	B[c]
BHC	7.2	19.5	21.0	24.0	25.9
Malathion	8.7	19.1	20.9	24.6	26.9
Untreated	8.1	17.9	19.4	21.9	23.8

[a] Indicates proportion of nitrogen per average protein.
[b] As received at the analysis laboratory.
[c] On a dry or moisture-free basis.

table 2. Protein content was almost 2% lower in the samples from the untreated plots, heavily infested with aphids the previous fall. Carotene was also lower in the untreated plot by approximately 3 milligrams per 100 grams, compared with samples from the two treated plots.

It may be concluded from the results of this experiment that although treatment increased quality of hay slightly, there was little or no increase in quantity. Therefore, the cost of spraying to control fall infestations of spotted alfalfa aphids on established stands of alfalfa for the benefit of the following spring crop is not warranted unless the grower is particularly concerned about obtaining a high quality alfalfa.

References Cited

Parker, Roy V., Vernon E. Burton, and Ray F. Smith. 1956. Aphid damage to alfalfa hay. California Agric. 10(3):5.

Sorensen, E. L., C. C. Burkhardt, and Wayne Fowler. 1958. Alfalfa seed production in Kansas. Kansas Agric. Expt. Sta. Circ. **290:** 11–13.

Studies on the Mode of Action of Synergized Bayer 21/199[1] and Its Corresponding Phosphate in the House Fly[2]

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Abstract

The toxicity and the *in vitro* and *in vivo* anticholinesterase activity of Bayer 21/199, more recently called Co-Ral® (*O*-(3-chloro 4-methylumbelliferone) *O,O*-diethyl phosphorothioate), and its corresponding phosphate, with and without piperonyl butoxide, have been studied in the house fly (*Musca domestica* L.). Bayer 21/199 and its corresponding phosphate were isotoxic when administered topically to flies. Both compounds were approximately 2.8 times more toxic when administered jointly with piperonyl butoxide. The phosphate of 21/199 was a 435 times more potent anticholinesterase agent *in vitro*. No increase in inhibition was found with the addition of piperonyl butoxide *in vitro*, but this compound markedly synergized the anticholinesterase activity of 21/199 *in vivo*. Bayer 21/199 apparently was converted to its corresponding phosphate *in vivo* and piperonyl butoxide did not appear to be associated with its oxidative metabolism. A reversibility in cholinesterase inhibition occurred in flies treated with 21/199 with or without piperonyl butoxide.

Although most of our knowledge on pesticide synergism has been concerned with pyrethrum and allethrin (Schmidt 1955) and halogen-containing insecticides (Summerford 1954), several workers have reported the synergism of organophosphorus insecticides. In studies with the body louse (*Pediculus humanus humanus* L.) Eddy *et al.* (1954) observed that synergists effective in combination with Bayer 21/199 (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate), Bayer 21/200 (*O*-(3-chloro-4-methylumbelliferone) *O,O*-dimethyl phosphorothioate), and Potasan® (*O*-(4-methylumbelliferone)*O,O*-diethyl phosphorothioate) were ineffective when used with parathion, malathion, and EPN. Hoffman *et al.* (1954) reported that most chemicals known to synergize pyrethrum also improved the effectiveness of certain organophosphorus insecticides, but some of the synergists were more effective with certain toxicants than with others. Bayer 21/100, 21/200, and Potasan were synergized more than the other toxicants tested. Rai *et al.* (1956) found that piperonyl butoxide antagonized the lethal action of malathion on two strains of house flies (*Musca domestica* L.), but synergized Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) and Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate).

The opposite effects of piperonyl butoxide on different organophosphorus insecticides suggest differences in their mode of action. Compounds of this group, generally believed to have similar modes of action, are toxic to animals because they interfere in the normal mechanism of nerve-impulse transmission through irreversible inhibition of the enzyme acetyl cholinesterase (Metcalf 1955, pp. 275–81; Nachmanson & Wilson 1951).

Among the many organophosphorus compounds tested, those showing the greatest degree of synergism were the *O*-(3-chloro-4-methylumbelliferone) or *O*-(4-methylumbelliferone) esters of *O,O*-dialkyl phosphorothioic acid. Bayer 21/199 is a member of this group. This insecticide may be effective against encysted cattle grubs (Smith & Richards 1954, Roth & Eddy 1955) or against migrating cattle grubs when applied as a spray (Brundrett *et al.* 1957).

This study was undertaken to gain a better understanding of the mode of action of organophosphorus

[1] Since these studies were completed the name of this material was changed to Co-Ral®.

[2] Accepted for publication January 23, 1959. Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the Master of Science degree in entomology, Oregon State College, Corvallis.

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synergism in which the pyrethrum synergist piperonyl butoxide ((α[2-(2-*N*-butoxyethoxy)ethoxy]-4,5-methylenedioxy-2-propyltoluene) was used in combination with Bayer 21/199 and the corresponding phosphate, *O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphate (0–21/199) in the house fly.

MATERIALS AND METHODS.—A strain of house flies moderately resistant to DDT was used in all these tests. The LD-50 and LD-90 were 1.2 and 100 micrograms of technical DDT per fly, respectively. Standard laboratory methods were employed to rear and maintain the fly colony. The rearing and holding rooms were maintained at 27 ± 2° C. and a relative humidity of 45–50%. All tests except *in vitro* cholinesterase (ChE) studies were conducted at the same temperature. Only 3- to 4-day-old female flies were used.

Twenty-five randomly selected flies anesthetized by carbon dioxide were treated on the mesonotum with a microsyringe calibrated to deliver 1 microliter of acetone per fly. The moribund and/or dead flies were recorded 24 hours after treatment. The LD-50's of the insecticides were determined by plotting the mean per cent mortalities converted to probits versus log dosage.

Bayer 21/199, 0–21/199, and 3-chloro-4-methylumbelliferone were tested with and without piperonyl butoxide at a 1:10 ratio. Piperonyl butoxide was also tested alone as a toxicant.[4]

The colorimetric method of Hestrin (1949) for acetylcholine (ACh) was used to determine ChE activity. For *in vitro* ChE studies groups of flies were anesthetized and decapitated. Their heads were homogenized for 5 minutes in a Potter-Elvehjem glass homogenizer with 5 ml. of 0.134 M phosphate buffer (pH 7.2) per 25 heads. "Merthiolate" (Thimerosol, Lilly), utilized by Swell & Treadwell (1950) to prevent the bacterial decomposition of tissue homogenates, was employed in the *in vitro* studies. A concentration of 0.075 ml. of a 1:10,000 solution per milliliter of fly-head brei stabilized the refrigerated homogenate up to 1 week without affecting the enzyme activity.

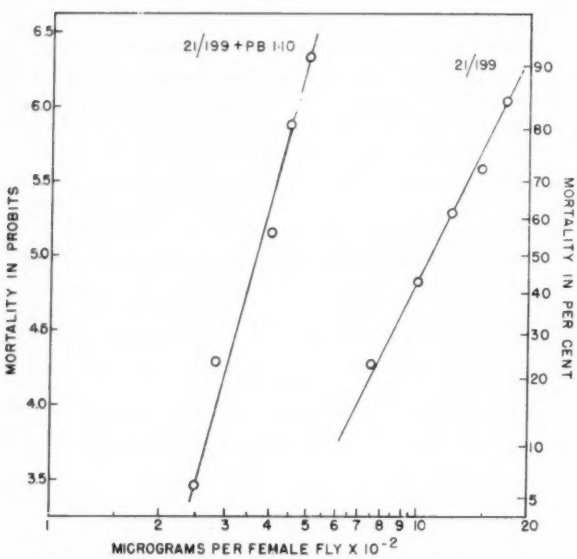

FIG. 1.—Toxicity to house flies of 21/199 with and without piperonyl butoxide.

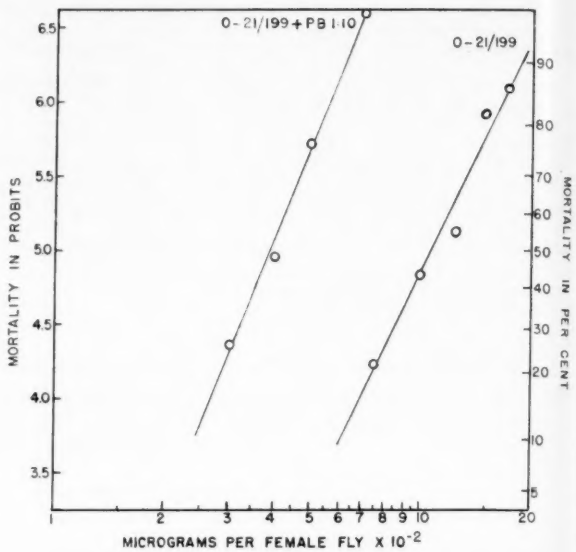

FIG. 2.—Toxicity to house flies of 0–21/199 with and without piperonyl butoxide.

A linear relationship between the quantity of brei employed and enzyme activity was found to exist until 4.40 micromoles of ACh had been hydrolyzed. The concentration of fly brei used in these tests was adjusted to give 3.00 ± 5% micromoles of ACh hydrolyzed (5 micromoles per fly head per hour) in a 15-minute control reaction period at 37° C. after the addition of 0.1 ml. of acetone. This is in good agreement with the findings of Wolf & Smallman (1956) and Metcalf & March (1950).

One milliliter of buffer solution and 0.7 ml. of brei were incubated at 37° C. with 0.1 ml. of an acetone solution of the inhibitor for 15 minutes. At this time 0.2 ml. of substrate (0.05 M acetylcholine chloride—5×10^{-3} M final concentration) was added and the mixture incubated for an additional 15 minutes. The reaction was then stopped and the remaining ACh determined colorimetrically. Acetone alone was used in the controls.

The IN-50's were calculated in the same manner as the LD-50's. Bayer 21/199, 0–21/199, and 3-chloro-4-methylumbelliferone were tested *in vitro* with and without piperonyl butoxide at a 1:10 ratio. Piperonyl butoxide was also tested alone as an anticholinesterase agent.

For *in vivo* ChE studies the flies were treated by microdrop in groups of 25 per concentration of toxicant. Two doses of Bayer 21/199 and 0–21/199 (0.0325 and 0.045 microgram per fly) with and without piperonyl butoxide 1:10 were employed. Only the toxicants plus piperonyl butoxide had previously been found to cause significant mortality. The toxicants alone caused only 4 to 8% mortality. After a predetermined interval flies were decapitated and their heads rinsed in three 50-ml. beakers containing acetone, in order to remove any toxicant from

[4] Bayer 21/199, 0–21/199, and the 3-chloro-4-methylumbelliferone were purified samples supplied by Geary Research Laboratory, New York, New York. The piperonyl butoxide was an analytical standard received from Fairfield Chemical Company, Baltimore Maryland.

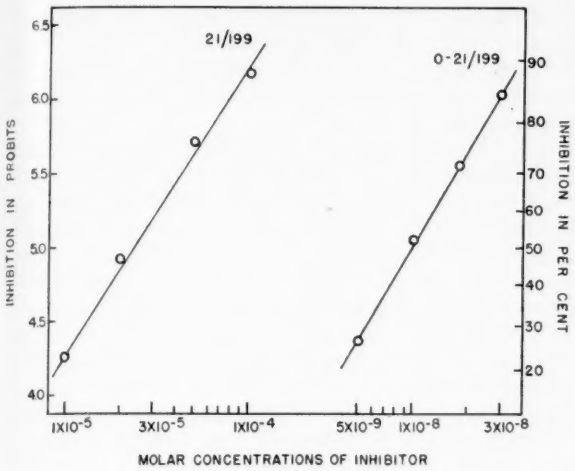

FIG. 3.—*In vitro* inhibition of fly head ChE by 21/199 and 0–21/199.

the exterior of the head capsules. After the acetone had evaporated, the heads were homogenized in 5 ml. of buffer solution. One group of flies was treated with acetone to serve as a control. A mixture of 0.5 ml. of brei and 1.3 ml. of buffer solution was incubated with 0.2 ml. of ACh at 37° C. for exactly 15 minutes. The reaction was then stopped and the ACh remaining was determined colorimetrically. The control activity, based on 14 groups of 25 flies, was $3.00 \pm 12\%$ micromoles of ACh hydrolyzed. Four groups of flies were treated with each dose per interval of time, and the breis tested in duplicate for ChE activity.

RESULTS.—*Toxicity.*—Bayer 21/199 and 0–21/199 were isotoxic to house flies, the LD-50 of both compounds being 0.107 microgram per fly (figs. 1 and 2). The respective LD-50's for the compounds plus piperonyl butoxide were 0.037 and 0.039 microgram per fly. Thus the synergized toxicants were 2.8 times more toxic than the toxicants alone.

The 3-chloro-4-methylumbelliferone was nontoxic at 10 micrograms per fly, whether used alone or with piperonyl butoxide. Piperonyl butoxide alone at 10 micrograms per fly was also nontoxic.

In vitro Studies.—The IN-50 of Bayer 21/199 with and without piperonyl butoxide was 2.3×10^{-5} M (figs. 3 and 4). The IN-50 of 0–21/199 was 1×10^{-8} 0–21/199 plus piperonyl butoxide was slightly more potent than 0–21/199 alone, with an IN-50 of 8×10^{-9} M. The 0–21/199 with and without piperonyl butoxide exhibited 435 times more anticholinesterase activity than the parent compound.

Piperonyl butoxide, when tested at a concentration 25 times greater than the maximum used with the toxicants, did not inhibit fly ChE. The 3-choloro-4-methylumbelliferone was a very poor anticholinesterase agent for fly-head ChE, with an IN-50 of 6×10^{-3} M. Piperonyl butoxide did not increase its anticholinesterase activity.

In vivo Studies.—The results of the *in vivo* ChE studies are presented in table 1 and figures 5 and 6. At the lower dosage the ChE activity compared with that of the control flies 1 and 2 hours after treatment was about the same for 21/199 with or without piperonyl butoxide. After 2 hours the mean knockdown was 12% for 21/199 plus piperonyl butoxide, but no appreciable knockdown was observed for 21/199 alone.

After 2 hours there was very little increase in inhibition in the flies treated with 21/199 alone. However, when piperonyl butoxide was added, the ChE activity continued to decrease until at 6 hours it was only 44%, or about half of that found at 2 hours. Fifty-nine and 44% knockdown occurred at 4 and 6 hours, respectively, in the flies treated with the synergized toxicant. At these intervals less than 3% of the flies treated with 21/199 alone were severely affected. Compared with the 6-hour interval, the flies sacrificed at 12 hours exhibited less knockdown and ChE inhibition following treatment with both the synergized and unsynergized toxicant.

At 0.045 microgram per fly the addition of piperonyl butoxide appeared to increase the anticholinesterase activity even at the early intervals. The minima in control activity, 48% for 21/199 alone and 28% with piperonyl butoxide, were found in the flies sacrificed at 2 hours. At this time maximum knockdown (9%) occurred with 21/199 alone. The maximum knockdown for synergized 21/199 (83%) was found after both 4 and 6 hours. During these intervals the ChE activity was approximately the same as that found after 2 hours. However, with 21/199 alone ChE inhibition in the flies sacrificed at 4 and 6 hours was only about half that found after 2 hours. Compared with the 6-hour interval, less knockdown and ChE inhibition occurred in flies 12 hours after treatment with the synergized and unsynergized toxicant.

Preliminary *in vivo* studies with 0–21/199 gave widely varying results. At the lowest dose tested, 0.030 microgram per fly, the ChE inhibition after 1 hour was 51–75% for the toxicant alone and 92–100% when piperonyl butoxide was added. No flies were knocked down at this dose.

DISCUSSION.—Although 0–21/199 was 435 times more potent than 21/199 as an anticholinesterase agent against house fly head ChE *in vitro*, the two compounds were equally toxic to the flies. When piperonyl butoxide was administered jointly with either compound, it did not increase the anticholinesterase activity *in vitro*, but did in-

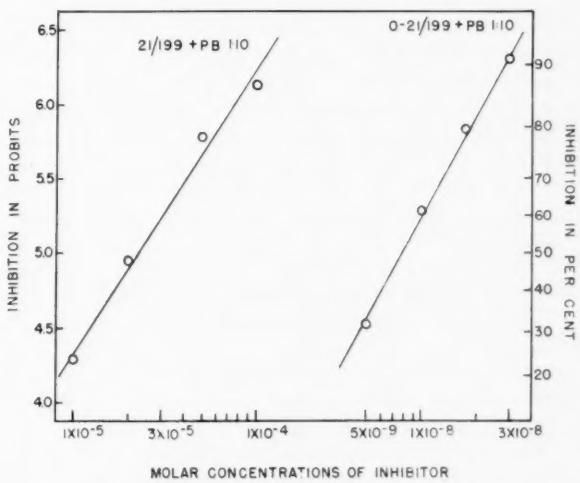

FIG. 4.—*In vitro* inhibition of fly head ChE by 21/199 and 0–21/199 plus piperonyl butoxide.

Table 1.—Knockdown and control ChE activity at intervals following application of Bayer 21/199 with and without piperonyl butoxide (1:10).

Hours	Without Synergist		With Synergist	
	Per Cent Knockdown	Per Cent of Control Activity	Per Cent Knockdown	Per Cent of Control Activity
	0.0325 microgram per fly			
1	0	80 ± 7	0	84 ± 7
2	2 ± 2	73 ± 7	12 ± 3	74 ± 1
4	3 ± 3	87 ± 4	59 ± 4	59 ± 8
6	2 ± 3	67 ± 5	44 ± 5	44 ± 10
12	0	81 ± 3	27 ± 13	60 ± 3
	0.045 microgram per fly			
1	1 ± 2	76 ± 10	2 ± 2	66 ± 9
2	9 ± 3	48 ± 5	44 ± 3	28 ± 17
4	2 ± 3	73 ± 6	83 ± 4	28 ± 6
6	5 ± 2	66 ± 9	82 ± 7	29 ± 3
12	2 ± 3	79 ± 8	78 ± 2	43 ± 12

crease it markedly *in vivo*. Concurrently the toxicity of both compounds was increased 2.8 times when administered with piperonyl butoxide. From these data it appears that the parent compound is either nontoxic or slightly toxic to house flies and is converted to its corresponding phosphate *in vivo*.

The *in vivo* and *in vitro* conversion of a phosphorothioate to its corresponding phosphate has been demonstrated for several pesticides (Casida 1956), including Bayer 21/199 (Robbins *et al.* 1959). Since the toxicity of both 21/199 and its corresponding phosphate can be increased by the addition of piperonyl butoxide, the mode of action of this synergist does not seem to be associated with the oxidative metabolism of this phosphorothioate.

Further data to support the theory that 21/199 is converted to 0–21/199 was obtained in preliminary tests with 0–21/199 *in vivo*. Only 5% of a nontoxic dose (0.050 microgram per fly) present in either the haemolymph or the cuticle of the head was sufficient to inhibit approximately 50% of the enzyme ChE *in vitro*, and also to mask the true *in vivo* activity of the compound. Nearly 100 times the nontoxic dose of 21/199 (0.045 microgram per fly) would have to be present in the same conditions to inhibit only 23% of the enzyme *in vitro:* yet 53% inhibition occurred at this dose *in vivo* after 2 hours.

The *in vivo* inhibition data of fly head ChE indicated that apparent rapid recovery or the reversibility of enzyme inhibition occurs with 21/199. The data also show that fewer flies are knocked down after 12 hours than after 6 hours. Return of ChE activity after inhibition by organophosphorus compounds has been demonstrated by Davison (1955). This worker states that the stability of the phosphorylated enzyme depends upon the enzyme inhibited and the organophosphorus compound used.

The 3-chloro-4-methylumbelliferone was nontoxic to house flies and could not be synergized with piperonyl butoxide, and was a poor anticholinesterase agent. Structurally related coumarin has been reported to be an anticholinesterase agent (Hase & Mizuno 1952).

Organic phosphorothioates are generally less susceptible to hydrolysis than the corresponding phosphates, the latter being better phosphorylating agents and therefore more potent *in vitro* anticholinesterase agents (Casida 1956, Metcalf 1955, pp. 275–81). Therefore, the difference between the *in vivo* and *in vitro* activities of 21/199 and 0–21/199 may be due either to differences in rates and routes of absorption or to their relative stability in the biological system.

The ChE of the nervous system is a final site of action of organophosphorus compounds (Augustinsson 1948, Nachmansohn & Wilson 1951). However, synergism does not have to occur at the site of action, but the synergist may increase the biological activity of the toxicant by competing with it for sites of loss through nonspecific absorption or by inhibiting and/or competing with the enzyme sites which are active in the detoxification of the toxicant. This theory, postulated by Veldstra (1956), contains a complex but acceptable biochemical explanation for a possible mode of action of organophosphorus synergism. A toxicant administered to an organism undergoes various processes of metabolism and elimination, such as oxidation to the oxygen analog, phosphorylating reactions at the final site of action, hydrolysis to more polar degradation products, and excretion. When a toxicant is administered alone, only a part of the compound is available to phosphorylate the ChE enzyme at the final site of action before it is hydrolyzed or excreted. If a synergist is administered jointly with the toxicant, it can be assumed that both compounds are competing for identical sites of loss, and thereby more of the toxicant will remain in the organism longer and, if necessary, undergo a hypertoxic change, and ultimately more of it will phosphorylate the ChE at the final site of action. Thus, a higher mortality would result.

Such a theory may be invalid, because not all organophosphorus toxicants can be synergized. However, compounds capable of marked synergism contain similar structures, such as umbilliferone in Bayer 21/199, 21/200, and Potasan. Therefore, a correlation between the structure of the synergist and that of the toxicant may exist before synergism occurs. Such a correlation in regard to competition for sites of loss would explain in part why some pesticides are more readily synergized than others.

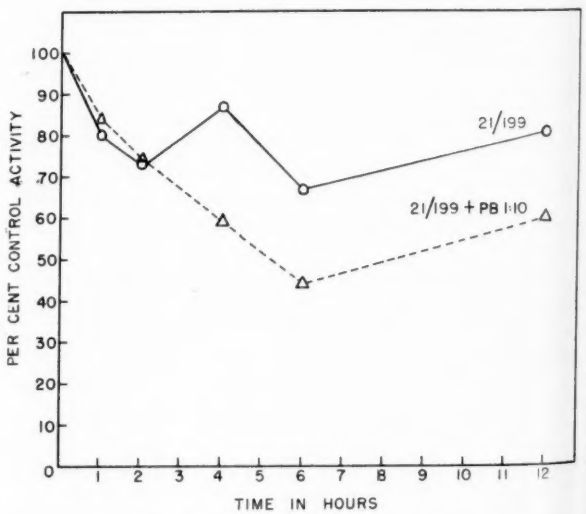

FIG. 5.—*In vivo* inhibition of fly head ChE by 0.0325 microgram of 21/199 per fly with and without piperonyl butoxide.

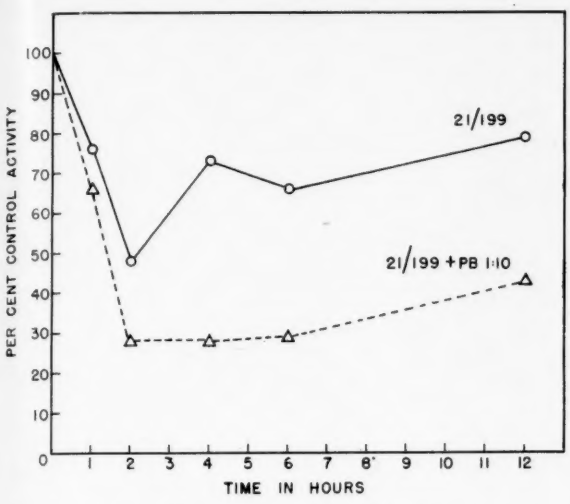

FIG. 6.—*In vivo* inhibition of fly head ChE by 0.045 microgram of 21/199 per fly with and without piperonyl butoxide.

The inhibition of the esterase responsible for the detoxification of pyrethrum by hydrolysis of the ester linkage has been postulated as a possible mode of action of piperonyl butoxide in pyrethrum synergism (Chamberlain 1950). In studies with P^{32}-labeled 21/199 in the mouse, Robbins *et al.* (1959) found that joint oral administration with piperonyl butoxide in some manner prevents the enzymic hydrolysis of 21/199 to more polar degradation products.

This theory was advanced because more of the unchanged toxicant was found in most of the tissues studied when the compound was administered jointly with piperonyl butoxide. The radioactive compounds found in mice treated with 21/199 alone were mainly more polar degradation products. It is possible that the toxicant was competing with the synergist for sites of loss in the mice treated with 21/199 plus piperonyl butoxide, but was more easily metabolized by mice treated with 21/199 alone.

REFERENCES CITED

Augustinsson, Klas-Bertil. 1948. Cholinesterases, a study in comparative enzymology. Acta Physiol. Scand. **15:** 1–182.

Brundrett, H. M., W. S. McGregor, and R. C. Bushland. 1957. Systemic cattle grub control with Bayer 21/199 sprays. Agric. Chemicals **12:** 36–7, 123–5.

Casida, John E. 1956. Metabolism of organophosphorus insecticides in relation to their antiesterase activity, stability, and residual properties. Jour. Agric. and Food Chem. **4:** 772–85.

Chamberlain, Roy W. 1950. An investigation on the action of piperonyl butoxide with pyrethrum. Amer. Jour. Hyg. **5:** 153–83.

Davison, A. N. 1955. Return of cholinesterase activity in the rat after inhibition by organophosphorus compounds. 2. A comparative study of true and pseudo cholinesterase. Biochem. Jour. **60:** 339–46.

Eddy, Gaines W., M. M. Cole, and A. S. Marulli. 1954. Louse powder synergists. Soap and Chem. Spec. **30:** 121–3, 143–5.

Hase, Eiji, and Yoshihisa Mizuno. 1952. Studies on the mechanism of action of ChE. III. Kinetic studies on the inhibitory action of coumarin and several alkylamonium compounds upon cholinesterase. Jour. Biochem. **39:** 273–86.

Hestrin, Shlomo. 1949. The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. Jour. Biol. Chem. **180:** 249–61.

Hoffman, Robert A., T. L. Hopkins, and A. W. Lindquist. 1954. Tests with pyrethrum synergists combined with some organic phosphorus compounds against DDT-resistant flies. Jour. Econ. Ent. **47:** 72–6.

Metcalf, Robert L. 1955. Organic insecticides, their chemistry and mode of action. 392 pp. Interscience Publishers, New York.

Metcalf, Robert L., and Ralph B. March. 1950. Properties of acetylcholine esterases from the bee, the fly and the mouse and their relation to insecticidal action. Jour. Econ. Ent. **43:** 670–7.

Nachmansohn, David, and Irwin B. Wilson. 1951. The enzymic hydrolysis and synthesis of acetylcholine. Advances Enzymol. **12:** 259–339.

Rai, Lallan, Saad E. D. Afifi, H. C. Fryer, and C. C. Roan. 1956. The effect of different temperatures and piperonyl butoxide on the action of malathion on susceptible and DDT-resistant strains of house flies. Jour. Econ. Ent. **49:** 307–10.

Robbins, William E., Theodore L. Hopkins, and Darrell I. Darrow. 1959. Synergistic action of piperonyl butoxide plus Bayer 21/199 and its corresponding phosphate in mice. (In press).

Roth, A. R., and Gaines W. Eddy. 1955. Tests with some phosphorus compounds against cattle grubs. Jour. Econ. Ent. **48:** 201–2.

Schmidt, Claude H. 1955. Studies of synergists for pyrethrins and related synthetic compounds. Paper presented before North Central Branch of Ent. Soc. America, March 24–25, 1955.

Smith, Charles L., and Rowland Richards. 1954. New insecticides for control of the cattle grub. Jour. Econ. Ent. **47:** 712–3.

Summerford, W. T. 1954. Synergism and synergists. Review of synergism among halogen-containing insecticides and halogen-containing synergists. Jour. Agric. and Food Chem. **2:** 310–27.

Swell, Leon, and C. R. Treadwell. 1950. Cholesterol esterases. II. Characterization of the esterifying cholesterol esterase of pancreatine. Jour. Biol. Chem. **182:** 479–87.

Veldstra, H. 1956. Synergism and potentiation with special reference to the combination of structural analogues. Pharmacol. Rev. **8:** 339–87.

Wolf, L. S., and B. N. Smallman. 1956. The properties of cholinesterase from insects. Jour. Cell. and Comp. Physiol. **48:** 215–36.

Ronnel and Co-Ral for Horn Fly Control on Cable Type Back Rubbers[1]

EDWARD C. BURNS,[2] S. E. MCCRAINE,[3] and D. W. MOODY[3]

ABSTRACT

Co-Ral (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) at 0.25% and ronnel at 1% oil solution were tried as back-rubber formulations for horn fly (*Siphona irritans* (L.)) control. Five per cent toxaphene was used as the standard of comparison. Both the phosphate insecticides compared favorably with toxaphene for horn fly control in cable type back rubbers. Rondel was formerly designated Korlan®, Trolene®, or Dow ET-57.

An increasing number of cattlemen are expressing concern with the poor results that they are obtaining with the chlorinated hydrocarbon insecticides for horn fly (*Siphona irritans* (L.)) control. This situation causes concern because of the possibility of resistance, in which case it will be necessary to change to different type compounds such as the organic phosphates. At the present time few of the phosphates are approved for topical application.

MATERIALS AND METHODS.—The cable type back rubber, Rogoff & Moxon (1952), was used in the evaulation of ronnel (formerly designated Korlan®, Trolene® or Dow ET-57) and Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) for horn fly control. Four herds of cattle were treated with each of the phosphate compounds while a 5% solution of toxaphene was used on four herds as a standard of comparison. At the beginning of the experiment, the rubbers were treated with 6 quarts of solution. A 2 to 1 mixture of diesel oil and nondetergent 30 SAE motor oil was used as the carrier.

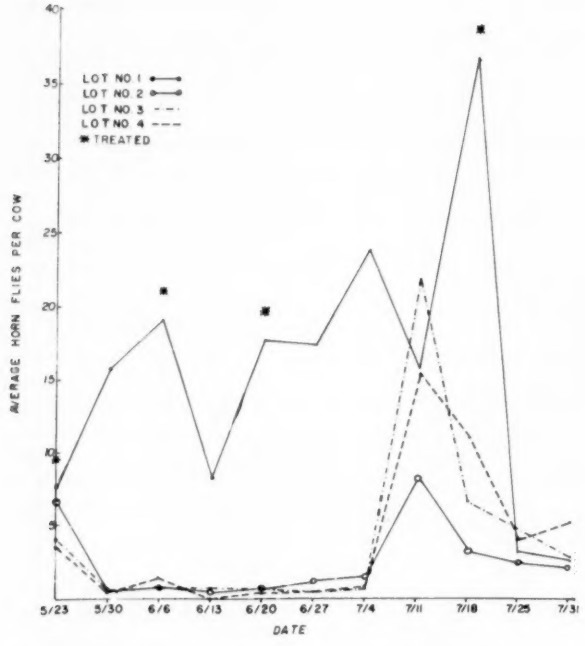

FIG. 1.—Horn fly control in four herds of beef cattle using cable type back rubbers treated with 5% toxaphene.

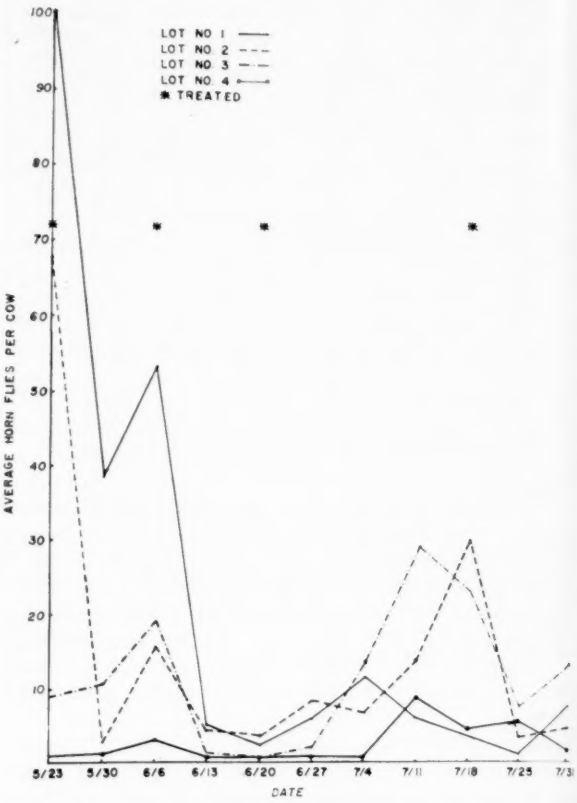

FIG. 2—Horn fly control in four herds of beef cattle using back rubbers treated with 0.25% Co-Ral.

It was found that this mixture was too volatile, and beginning June 20 the mixture was changed to 2 to 1 motor oil and diesel fuel. Rubbers were charged routinely every 2 weeks through June 20, after which they were not treated again for 1 month.

Three of the lots consisting of one Co-Ral, one toxaphene and one ronnel treatment contained approximately 40 acres. One of the Co-Ral lots contained about 80 acres. Herd size ranged from 25 to 40 head. These pastures were in the same general area. The eight remaining lots were approximately 1 mile from the large pastures. They were 6 acres in size and contained 8 to 10 cattle per lot. All the pastures were open, but with an occasional shade tree, and the rubber or rubbers were located in the most frequented areas.

Three-gallon Hudson type hand sprayers were used for mixing and applying the formulations. Toxaphene was applied at 5%, ronnel at 1%, and Co-Ral at 0.25% solution. Because of the poor solubility of 25% Co-Ral wettable powder in diesel fuel, it was premixed in the heavy oil.

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Horn fly counts were made by selecting at random 10 cows in the herd, except in the small herds where all animals were counted, and counting all the horn flies on one side of the animals. When the weekly fly counts were made the herds were counted in the same order to avoid error caused by effect of time of day on horn fly numbers, Raun & Casey (1956).

RESULTS AND DISCUSSION.—The performance of the three materials is shown in figures 1, 2, and 3. Horn flies in the small lots of cattle with toxaphene-treated back rubbers were held to low numbers when the back rubbers were treated semimonthly. However, numbers increased sharply when applications were prolonged beyond 2 weeks. In the large lot of cattle, fly numbers were not brought to a satisfactory level until near the end of the experiment. This was the only brahman herd of cattle included in the experiment and the animals seemed reluctant to use the device. Co-Ral at 0.25% effectively reduced high populations of horn flies, but populations tended to increase when the rubbers were not treated on a 2-week basis. One per cent ronnel was effective in reducing populations to a minimum. Although fly numbers

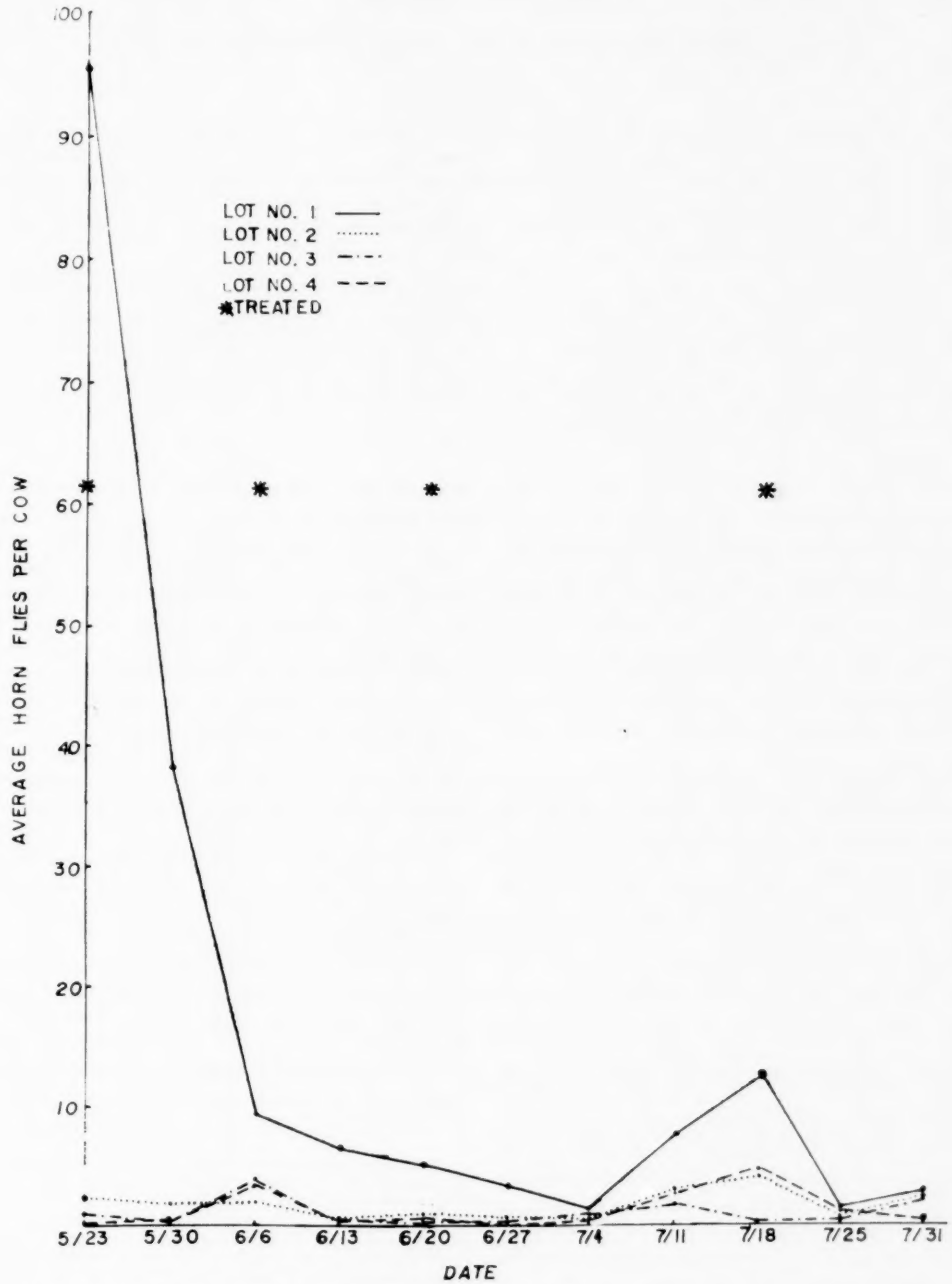

FIG. 3.—Horn fly control in four herds of beef cattle using back rubbers treated with 1% ronnel.

began increasing 2 weeks following treatment of the rubbers, populations were maintained at lower levels than those of the other treatments.

REFERENCES CITED

Raun, E. S., and D. J. Casey. 1956. A comparison of back rubber formulations for controlling horn and stable flies in Iowa. Jour. Econ. Ent. **49**(3): 395–7.

Rogoff, W. M., and A. L. Moxon. 1952. Cable type back rubbers for horn fly control on cattle. Jour. Econ. Ent. **45**(2): 329–34.

Effects of Thimet and Various Stickers on Germination and Seedling Growth of Wheat[1]

JAMES R. GIFFORD, C. C. BURKHARDT, and H. W. SOMSEN[2]

ABSTRACT

Seed treatments to determine the effects of Thimet® (*O,O*-diethyl *S*-(ethylthio) methyl phosphorodithioate) with various oils and chloranil on germination, emergence, and seedling survival of Bison, Ponca, and Pawnee varieties of wheat were conducted at Manhattan, Kansas, in 1957. Oils of soybean, peanut, corn, and rice, and water were used as stickers.

Generally no adverse effects were indicated on germination or emergence resulting from treatment with as high as 1 pound Thimet plus water. Seedling survival, however, appeared to be reduced by treatment with ¾ and 1 pound Thimet plus water.

Germination of the three varieties was affected differently by the various oils. Thimet at all rates, when used with the oil stickers, reduced germination. However, a difference was noted in the response of each variety to various treatments. An increase in germination when chloranil, a fungicide, was used with the oils counteracted a reduction in germination which occurred when oils were used alone. Chloranil did not counteract a highly significant reduction of germination resulting from treatment with oils plus Thimet.

With one exception, emergence and seedling survival were reduced greatly by treatment with oils plus Thimet. Survival was significantly increased by adding chloranil with oils and Thimet, but the increase was negligible from a practical standpoint because of the highly significant reduction of the survival found for all treatments when Thimet was used with oils.

The treatment of seed with systemic insecticides has become increasingly important in combating insect pests on seedlings of various crops. Insecticides are easily applied and relatively small quantities are needed. Because of large acreages and relatively low income return per acre for wheat, seed treatment is economically more feasible than other methods of combating many wheat insects. Because some systemic insecticides are phytotoxic to both seeds and seedlings, preliminary tests were made to determine effects on germination, emergence, and subsequent growth.

METHODS AND MATERIALS.—EXPERIMENT I.—Greenhouse tests were conducted in 1957 to determine effects of Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate) seed treatment, alone and with natural oil stickers, on germination and emergence. Bison, Ponca, and Pawnee wheat were treated with oils of soybean, peanut, and corn, and water, alone and with various rates of Thimet 44D (½, ¾, and 1 pound actual per 100 pounds seed). Oils used were 2% and water 1% by weight. The seed was treated in containers about five times the volume of the seed by tumbling 20 minutes at approximately 50 r.p.m. on a rotating machine with a 10-inch radial swing.

Germination.—Effects of treatment were determined 0, 1, 2, 5, and 8 weeks after treatment; 5 replications of 10 seeds each were germinated for each treatment and check. Seeds were placed between double thicknesses of moistened paper towels, lying in sterilized vermiculite flats saturated with water. Germination counts were made 7 days after placing the seeds on the pads.

Estimated Survival.—Seedling survival, based on appearance and subsequent growth, was evaluated 10 days after planting. Seeds supporting healthy shoot and root systems were considered potential plants.

Emergence and Subsequent Growth.—At each of the previously given intervals, 10 seeds of each treatment and checks were planted in soil flats. Emergence counts for each treatment were taken 8 days from planting date.

EXPERIMENT II.—Data from Experiment I indicated that oils used alone or in combination with Thimet reduced germination and survivial of seedlings. Molds commonly occurred in the germination flats of Experiment I and may have reduced germination, so Experiment II was conducted to determine effects of a fungicide applied with the various treatments.

Basically, procedures in this experiment were the same as for Experiment I. However, only Ponca and the 1-pound rate of actual Thimet per 100 pounds of seed were used. Treatments consisted of Thimet 44D used alone and in combination with water, and oils of soybean, corn, peanut, and rice. The same treatments were repeated with chloranil (Spergon®) (tetrachloro-p-benzoquinone 96%) at 1 pound of formulated material per 100 pounds seed.

Germination and estimated survival evaluations were made 0, 2, 4, and 8 weeks after seed treatment on 4

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The authors acknowledge the help of Gary Krause with statistical analyses.

Table 1.—Percentages of germination of three wheat varieties following seed treatment, arrayed in descending order. Experiment I. Manhattan, Kansas. 1957.

PAWNEE				PONCA				BISON			
Treatment				Treatment				Treatment			
Sticker Water or Oil	Rate Thimet[a] (lb.)	Germination (%)	Significance[b]	Sticker Water or Oil	Rate Thimet[a] (lb.)	Germination (%)	Significance[b]	Sticker Water or Oil	Rate Thimet[a] (lb.)	Germination (%)	Significance[b]
Water	0	97.2		Check	0	97.8		Water	0	96.4	
Check	0	96.2		Water	0	97.2		Check	0	95.6	
Water	0.75	96.0		Water	0.5	96.0		Water	0.5	94.8	
Water	0.5	94.4		Water	0.75	94.0		Water	0.75	92.4	
Water	1	93.2		Peanut	0	92.8		Water	1	90.4	
Corn	0	85.2		Water	1	92.0		Corn	0	86.8	
Peanut	0	82.0		Corn	0	90.0		Soybean	0	83.6	
Soybean	0	78.4		Soybean	0	84.8		Peanut	0	83.6	
Peanut	0.5	74.8		Peanut	0.5	78.8		Peanut	0.5	52.4	
Soybean	0.75	74.4		Peanut	0.75	74.4		Peanut	0.75	46.4	
Soybean	0.5	73.6		Soybean	0.5	73.6		Soybean	0.5	46.4	
Corn	0.75	71.6		Corn	0.5	67.6		Peanut	1	46.0	
Corn	0.5	70.0		Soybean	0.75	64.0		Corn	1	43.6	
Peanut	1	69.6		Peanut	1	63.2		Corn	0.5	42.8	
Corn	1	68.8		Corn	1	62.8		Soybean	0.75	40.4	
Soybean	1	67.2		Soybean	1	60.4		Corn	0.75	32.0	
Peanut	0.75	63.6		Corn	0.75	59.6		Soybean	1	24.0	
L.S.D. at 5% level		9.2				8.7				13.2	

[a] Actual per 100 pounds seed.
[b] Means paralleled by the same line are not significantly different at P = .05.

replications of 25 seeds each in each of 2 environments. One set of 4 replicates was under winter greenhouse conditions (70 to 75° F.). The second set was in an attic room where night temperatures dropped as low as 40° F. and daytime temperatures seldom were higher than the lower 60's.

RESULTS AND DISCUSSION.—EXPERIMENT I.—*Germination.*—Because storage of seed up to 8 weeks after treatment had little or no adverse effect on germination, data of the different intervals were combined for analysis. Treatments of each variety, arrayed in descending order of germination are shown in table 1. Treatments not paralleled by the same line were significantly different at P = .05.

Thimet with a water sticker did not adversely affect germination. Germination in the oil treatments varied among varieties. All oils used alone significantly lowered germination of Pawnee. Only soybean oil reduced germi-

Table 2.—Percentage of seedling survival for three wheat varieties. Experiment I. Manhattan, Kansas. 1957.

PAWNEE				PONCA				BISON			
Treatment				Treatment				Treatment			
Sticker Water or Oil	Rate Thimet[a] (lb.)	Seedling Survival (%)	Significance[b]	Sticker Water or Oil	Rate Thimet[a] (lb.)	Seedling Survival (%)	Significance[b]	Sticker Water or Oil	Rate Thimet[a] (lb.)	Seedling Survival (%)	Significance[b]
Check	0	88.3		Water	0	96.0		Check	0	93.3	
Water	0	82.8		Check	0	88.3		Water	0	90.0	
Water	0.5	78.8		Water	0.5	86.0		Water	0.75	76.8	
Corn	0	71.5		Peanut	0	82.5		Water	0.5	75.3	
Water	0.75	69.5		Water	0.75	82.0		Corn	0	75.0	
Peanut	0	68.8		Corn	0	80.0		Peanut	0	73.0	
Water	1	68.0		Water	1	71.3		Water	1	71.3	
Soybean	0	64.8		Soybean	0	70.0		Soybean	0	71.3	
Corn	0.5	55.0		Peanut	0.5	67.8		Corn	0.5	38.5	
Soybean	0.75	52.0		Peanut	0.75	60.3		Peanut	0.5	34.0	
Peanut	0.5	51.5		Corn	0.5	58.3		Soybean	0.5	32.5	
Soybean	0.5	49.8		Soybean	0.5	56.0		Peanut	0.75	26.0	
Corn	0.75	46.5		Soybean	0.75	50.0		Peanut	1	26.0	
Corn	1	39.8		Peanut	1	49.8		Soybean	0.75	25.3	
Soybean	1	37.3		Soybean	1	45.8		Corn	1	24.3	
Peanut	0.75	33.5		Corn	1	42.5		Corn	0.75	18.8	
Peanut	1	28.5		Corn	0.75	42.0		Soybean	1	9.8	
L.S.D. at 5% level		12.73				11.49				11.04	

[a] Actual per 100 pounds seed.
[b] Means paralleled by the same line are not significantly different at P = .05.

nation of Ponca. None affected Bison.

Germination between varieties varied when oils were used in combination with different rates of Thimet. Adding Thimet to oils on Ponca and Bison significantly reduced germination below oils alone. Pawnee, however, was not adversely affected by peanut oil plus ½ pound Thimet, or soybean oil with as much as ¾ pound Thimet.

Germination of seed treated with 1 pound Thimet usually was not significantly below that of ½ pound used in combination with oils. Again varieties reacted differently to different oils with various rates of Thimet. With corn oil, no differences between rates of Thimet were detected in any variety. One pound of Thimet with peanut oil reduced germination below ½ pound in Ponca but not in Bison or Pawnee. With soybean oil significant differences were noted in germination of Ponca and Bison between higher and lower rates of Thimet.

Some varietal differences occurred when different oils were used with the same rate of Thimet. In Pawnee and Bison there was no difference in germination between any of the oil treatments used with ½ pound Thimet. Ponca had significantly lower germination when treated with corn oil and ½ pound Thimet than with peanut oil and ½ pound Thimet.

Corn oil and soybean oil plus ¾ pound Thimet significantly reduced Ponca germination below peanut oil plus the same rate. At ¾ pound Thimet only corn oil significantly reduced germination of Bison. Peanut oil treatments resulted in lower germination than soybean oil on Pawnee.

In Pawnee and Ponca there were no significant differences between oils with 1 pound Thimet; however, on Bison lower germination resulted for soybean oil than either peanut or corn oil.

Estimated Survival.—Because seedling vigor was not affected by seed storage after treatment, data for intervals were combined for analysis.

Although reaction of the three varieties to the different treatments often varied, all varieties followed a general trend (table 2).

Seed treatments with oils or the two higher rates of Thimet plus water generally reduced seedling survival. The ½-pound rate of Thimet with water as a sticker reduced Bison survival and approached margins of significance in Pawnee and Ponca.

Adding Thimet at any rate to oils significantly reduced survival below the oils alone in all varieties. A pronounced example was Bison with its reduction of survival ranging from 35 to 61%.

Seedling survival of all varieties generally was affected about the same by all oils used with ½ pound Thimet. However, with ¾ pound Thimet varietal differences occurred. No significant differences occurred between oils used in combination with ¾ pound Thimet on Bison, but corn oil and peanut oil reduced survival of Ponca and Pawnee, respectively. Survival of Ponca and Pawnee was affected equally by all oils plus 1 pound Thimet. Bison exhibited additional survival reduction when treated with soybean oil and 1 pound Thimet.

Emergence.—With exception of 1 pound Thimet plus water on Bison, treating seed with Thimet and water did not adversely affect seedling emergence (table 3). Of all oils alone, only peanut oil on Bison significantly reduced seedling emergence below the check.

Varietal responses varied with oils in combination with various rates of Thimet. Ponca and Pawnee did not differ significantly (5% level) for the same treatments; however, both differed from Bison.

Table 3.—Average number of seedlings emerging from the various treatments of three wheat varieties, arrayed in descending order.[a] Experiment I. Manhattan, Kansas. 1957.

Pawnee				Ponca				Bison			
Treatment				Treatment				Treatment			
Sticker Water or Oil	Rate Thimet[b] (lb.)	No. Seedlings Emerging	Significance[c]	Sticker Water or Oil	Rate Thimet[b] (lb.)	No. Seedlings Emerging	Significance[c]	Sticker Water or Oil	Rate Thimet[b] (lb.)	No. Seedlings Emerging	Significance[c]
Check	0	8.5		Check	0	10.0		Check	0	10.0	
Water	1	8.5		Water	0.5	9.8		Water	0.5	9.5	
Water	0.5	8.3		Water	0.75	9.5		Water	0	9.0	
Corn	0	8.0		Water	1	9.3		Water	0.75	8.8	
Water	0	7.8		Water	0	9.3		Soybean	0	8.8	
Water	0.75	7.8		Peanut	0	8.5		Corn	0	8.0	
Soybean	0	7.5		Soybean	0	8.0		Water	1	7.5	
Peanut	0	7.3		Corn	0	7.8		Peanut	0	6.5	
Peanut	0.5	7.0		Soybean	0.5	7.5		Peanut	0.5	5.3	
Corn	0.5	6.0		Peanut	0.75	7.5		Soybean	0.5	5.0	
Peanut	0.75	5.8		Peanut	0.5	6.5		Soybean	0.75	3.5	
Peanut	1	5.8		Peanut	1	5.5		Peanut	0.75	3.3	
Soybean	0.5	5.8		Corn	0.75	5.5		Peanut	1	2.5	
Soybean	0.75	5.8		Corn	1	5.0		Corn	0.5	2.0	
Corn	0.75	5.5		Soybean	1	4.8		Corn	0.75	2.0	
Corn	1	4.8		Soybean	0.75	4.5		Corn	1	1.8	
Soybean	1	4.5		Corn	0.5	4.3		Soybean	1	1.8	
L.S.D. at 5% level		1.90				1.90				1.90	

[a] From 10 seeds at 0-, 1-, 2-, and 5-week intervals after treatment.
[b] Actual per 100 pounds seed.
[c] Means paralleled by the same line are not significantly different at P = .05

Table 4.—Mean germination percentages of Ponca wheat from two environments following seed treatment, rrayed in descending order. Experiment II. Manhattan, Kansas. 1957.

LOW TEMPERATURES (40–60° F.)					GREENHOUSE TEMPERATURES (70–75° F.)				
Treatment[a]					Treatment[a]				
Sticker Water or Oil	Thimet	Chloranil	Germination (%)	Significance[b]	Sticker Water or Oil	Thimet	Chloranil	Germination (%)	Significance[b]
None		×	97.5		Water		×	97.5	
Water		×	96.9		None		×	97.4	
None			96.9		Water			96.5	
Water			96.4		Peanut		×	96.3	
None	×		94.6		None			96.0	
Water	×	×	94.6		Water	×	×	96.0	
None	×	×	94.0		Corn		×	95.5	
Peanut		×	93.4		Water	×		94.9	
Water	×		92.6		None	×	×	94.6	
Corn		×	91.5		None	×		94.1	
Rice		×	90.3		Soybean		×	94.0	
Soybean		×	86.1		Rice		×	92.9	
Peanut			83.3		Peanut			86.9	
Corn			82.1		Soybean			86.1	
Soybean			81.9		Corn			85.3	
Rice			81.2		Rice			84.1	
Rice	×		76.0		Rice	×	×	80.9	
Corn	×		72.5		Corn	×	×	80.5	
Peanut	×		72.3		Peanut	×	×	76.9	
Soybean	×	×	71.9		Rice	×		75.4	
Rice	×	×	70.1		Peanut	×		74.8	
Corn	×	×	70.0		Soybean	×	×	74.6	
Soybean	×		69.5		Corn	×		72.0	
Peanut	×	×	68.5		Soybean	×		71.1	

[a] Thimet and chloranil both at 1 pound actual per 100 pounds seed.
[b] Means paralleled by the same line are not significant at P = .05, as determined by Duncan's test.

Table 5.—Array of mean percentages of seedling survival of Ponca wheat from two environments. Experiment II. Manhattan, Kansas. 1957.

LOW TEMPERATURES (40–60° F.)					GREENHOUSE TEMPERATURES (70–75° F.)				
Treatment[a]					Treatment[a]				
Sticker Water or Oil	Thimet	Chloranil	Seedling Survival (%)	Significance[b]	Sticker Water or Oil	Thimet	Chloranil	Seedling Survival (%)	Significance[b]
Water		×	94.1		Water		×	94.0	
None		×	92.1		None		×	93.4	
None			89.0		None			91.5	
Water			87.3		Peanut		×	88.9	
Peanut		×	86.3		Soybean		×	88.6	
None	×	×	84.3		Water			87.8	
Water	×	×	82.6		Corn		×	87.6	
Corn		×	81.9		Rice		×	86.8	
Rice		×	80.5		Water	×		85.1	
Soybean		×	78.7		Water	×	×	82.1	
Water	×		76.5		None		×	78.9	
None	×		76.1		None	×		77.6	
Corn			68.1		Corn			74.8	
Peanut			65.9		Peanut			73.0	
Soybean			65.9		Soybean			69.6	
Rice			63.6		Rice			67.3	
Rice	×	×	52.1		Rice	×	×	57.5	
Soybean	×	×	51.4		Corn	×	×	56.1	
Corn	×	×	50.9		Soybean	×	×	52.0	
Peanut	×	×	49.7		Peanut	×	×	50.4	
Rice	×		40.0		Rice	×		44.1	
Soybean	×		39.4		Soybean	×		42.9	
Corn	×		37.2		Peanut	×		40.8	
Peanut	×		35.0		Corn	×		40.3	

[a] Thimet and chloranil both at 1 pound per 100 pounds seed.
[b] Means paralleled by the same line are not significant at P = .05, as determined by Duncan's test.

Peanut oil plus ½ pound Thimet on Pawnee seed was the only oil-plus-Thimet treatment which did not significantly reduce seedling emergence of all varieties below that of the check. Except for this treatment, all oils plus different rates of Thimet reduced emergence of all variety seedlings from 29 to 82.5%.

EXPERIMENT II.—*Germination.*—Results of this experiment (table 4) were generally the same for both environments and essentially substantiated Experiment I. Thimet 44D alone or in combination with water and/or chloranil did not reduce germination. Chloranil counteracted reduced germination resulting from oils alone. Except for corn oil, when temperatures were 70 to 75° F., chloranil did not counteract highly significant reduced germination from oils plus Thimet.

Estimated Survival.—Tests under both environmental conditions indicated Thimet, alone or in combination with chloranil, reduced survival (table 5). Adding chloranil to different oils usually counteracted reduced survival caused by oils. Adding chloranil with oils plus Thimet significantly increased survival, but not enough to offset the highly significant effects of Thimet used with oils. The two different environments gave variable results for some treatments. In tests where low temperatures retarded growth of seedlings adding chloranil with Thimet or water plus Thimet either significantly increased survival or approached margins of significance. Under greenhouse conditions, where temperatures were 70 to 75° F., no difference was detected by adding chloranil to treatments of Thimet alone or of water plus Thimet.

Alfalfa Seed Treatments for Spotted Alfalfa Aphid Control in Southwestern Arizona[1]

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ABSTRACT

Treatment of alfalfa seed with systemic insecticides provided control of the aphid (*Therioaphis maculata* (Buckton)) on seedlings for periods up to 10 weeks, depending upon the temperature. Di-syston® (*O,O*-diethyl S-2-(ethylthio)ethyl phosphorodithioate) gave best control, followed closely by Thimet® (*O,O*-diethyl*S*-(ethylthio)methyl phosphorodithioate), and lindane. The treatment did not affect germination in field plots, but caused some phytotoxicity in laboratory tests. Treated seed stored a month before planting apparently lost no toxicity. Soil type did not appear to affect the control obtained. The treatment caused no significant reduction in nodulation.

The spotted alfalfa aphid, *Therioaphis maculata* (Buckton), has been a major pest of alfalfa in the Southwest since its discovery in 1954 (Tuttle & Butler 1954). The damage caused by this aphid was described by Dickson *et al.* (1955), Reynolds & Anderson (1955), and Davis *et al.* (1957). Reynolds and Anderson reported that the damage was caused in part by toxins injected into the plant. They found that seedlings are especially susceptible and new stands can be severely damaged by only a few aphids. In insectary studies Nielson (1958) observed that one adult aphid, together with its offspring, could kill a plant in the cotyledon stage in 10 to 12 days.

This ability to destroy seedling alfalfa has caused a serious problem in the establishment of new stands and in the reseeding of old stands. Some fields in the Yuma, Arizona, area have had to be replanted several times. Repeated applications of insecticides to young seedlings gave good control of the aphid, but were expensive and frequently had to be delayed a week or longer because of high winds. These delays often enabled the aphids to build up to damaging numbers. Frequent irrigations to protect the young seedlings from sand storms and from desiccation during hot, dry weather also prevented the use of ground dusting and spraying machinery in fields. A more practical and economical method of aphid control is needed to protect the alfalfa seedlings.

In preliminary tests conducted at Yuma in 1954 and 1955 seed treatments with systemic insecticides gave the most effective and economical control of the aphid on alfalfa seedlings. In 1956 and 1957 more extensive tests were carried out to determine the most promising insecticides, the most effective and economical dosages, the best formulations, relative effectiveness on different types of soil, and the effect of the insecticide on germination and nodulation.

MATERIALS AND GENERAL METHODS.—Because of the small size of alfalfa seed and its hard, smooth coating, it is difficult to retain a sufficient amount of insecticide on the seed. Dobson & Watts (1957) reported that the use of starch as a sticker was only partially satisfactory. Davis *et al.* (1957) and Reynolds *et al.* (1957) found a seed adhesive containing methylcellulose improved adherence.

In the Yuma tests a 5% methylcellulose solution was used with dust and activated-charcoal formulations. The seed was first dampened with 20 to 30 mm. of this solution per pound. The higher rate was necessary when more than 5 pounds of seed were treated or when high rates of dust or activated charcoal were used. The formulations were then applied to the dampened seed in predetermined amounts and thoroughly mixed.

Although this method of application was fairly satisfactory, some problems were encountered. Lumps of seed were formed and had to be screened out. In addition, an excess of sticker delayed drying of the seed, while a deficiency caused poor adhesion that resulted in a loss of insecticide during planting.

Soaking the seed in dilute liquid insecticide formulations caused problems of drying, stickiness, and some pregermination, as was also experienced by other workers

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[2] In cooperation with the University of Arizona Agricultural Experiment Station, Yuma.

(Davis *et al.* 1957, Reynolds *et al.* 1957). Application of technical insecticides or emulsifiable concentrates proved satisfactory. Measured amounts were applied to the seed, mixed throroughly, and the seed dried by adding small amounts of pyrophyllite. The pyrophyllite need not be measured but merely added until the seed is dried. No lumps were formed, the seed was damp only a few minutes and the pyrophyllite prevented stickiness. The omission of methylcellulose and the substitution of the low-cost pyrophyllite for activated charcoal also effected a substantial saving in the cost of seed treatment.

Small amounts of seed, less than 5 pounds, were treated in gallon cans and larger amounts in a cement mixer, the drum being rotated at 14 to 18 revolutions per minute.

Most plantings were made on small plots replicated six times in a randomized-block design. The smallest plots were 4 by 8 feet, 2 feet apart with 6-inch rows planted by hand. A specially designed furrow rake was made to form the rows by cutting the tines of a pitchfork back to 4 inches and welding them to the middle of the flat side of a piece of strap iron 22 by 2 by $\frac{1}{8}$ inches. Four cutter-bar knife sections from a mower were bolted 6 inches apart to the lower edge of the piece of strap iron. The treated seed was shaken into the furrows from an ice-cream carton through a $\frac{3}{16}$-inch hole in the lid. Plots 10 by 150 feet were planted with a Van Brunt grain drill, as were large (5-acre) unreplicated plots. Flood-type irrigation was used in all except the 5-acre plots, where sprinklers were used.

Spotted alfalfa aphid populations were estimated by periodic counts of the wingless aphids on 10 to 25 seedlings or trifoliolate leaves selected at random on each plot, similar to the method suggested by Nielson (1957). Counts on trifoliolate leaves were practical only after the plants reached the 3- to 4-leaf stage. Because of the transitory habits of winged aphids, they were not included in the counts.

Germination tests were made by placing 50 alfalfa seeds on damp filter paper in petri dishes. Four replications were used. After 5 days at room temperature the germinated plants were counted.

EFFECTIVENESS OF DIFFERENT INSECTICIDES, FORMULATIONS, AND PLANTING DATES.—Two almost identical tests were made 33 days apart with four dust and three activated-charcoal formulations applied at the rate of 2 pounds of active ingredient per 100 pounds of seed. The insecticides used were Di-syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate), BHC, Bayer 23129 (*O,O*-dimethyl *S*-2 (ethylthio) ethyl phosphorodithioate), Diazinon® (*O,O*-diethyl *O*-2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), lindane, Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), and Trithion® (*S*(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate). The seed was treated on October 24. Half of it was planted for the first test, and the remaining portion reserved for the second test. During the first 3 weeks the mean temperature was 62° F. and conditions were good for rapid plant growth. Moderate aphid populations, as indicated on the untreated check plots, and few predators provided ideal conditions for the test. Germination was good in all plots except those planted with BHC-treated seed, where the stand was reduced 25 to 30%.

The results of the first test are shown in table 1. Counts made 38 days after planting showed that best results were obtained with Di-syston, followed by lindane, Thimet, and BHC. Diazinon and Trithion showed no control at these counts.

On November 26, when the second lot of seed was planted, the weather was slightly cooler. Mean temperature during the first 3 weeks was 57° F., and plant growth was slower than in the October planting. Although heavy aphid populations were present when the seedlings emerged, a good initial stand was noted. Results given in table 2 show that all the insecticides provided better control than in the October planting. For consistent effectiveness and long residual action, Di-syston was superior to the other insecticides. Storing the treated seed for more than a month had no apparent effect on germination or toxicity of any of the insecticides. It is of interest that all insecticides tested, including such nonsystemics as lindane, Trithion, and Diazinon, provided at least some protection for at least 3 weeks during cool weather. The systemic action of lindane on alfalfa was noted previously by Koehler & Gyrisco (1957).

In two similar tests various emulsifiable concentrates or technical insecticides were used. The seed was treated with Bayer 23129, Bayer 23453 (*O,O*-dimethyl *S*-2 (ethylsulfinyl)ethyl phosphorodithioate), 6-bromopiperonyl chrysanthemumate, and Thimet. Technical

Table 1.—Control of the spotted alfalfa aphid on alfalfa seedlings grown from seed treated with different insecticide dusts and activated-charcoal formulations.[a]

TREATMENT	AFTER DAYS INDICATED							
	23		30		38		52	
	No. of Aphids	Control (%)	No. of Aphids	Control (%)	No. of Aphids	Control (%)	No. of Aphids	Control (%)
Dusts:								
BHC 30%	38	63	82	47	332	43	—	—
Lindane 75%	10	90	30	81	185	68	—	—
Diazinon 25%	108	0	222	0	1090	0	—	—
Trithion 25%	87	16	146	6	756	0	—	—
Activated charcoal:								
Di-syston 50%	3	97	12	92	0	100	138	87
Thimet 44%	25	76	42	73	232	60	—	—
Untreated	103	—	156	—	582	—	1030	—

[a] Seed treated and planted October 24, 1956. Counts 23 and 30 days after planting based on 60 seedlings; later counts on 120 seedlings.

Table 2.—Control of the spotted alfalfa aphid on alfalfa seedlings grown from seed treated with different insecticide dusts and activated charcoal formulations.[a]

Treatment	After Days Indicated							
	25		31		45		52	
	No. of Aphids	Control (%)	No. of Aphids	Control (%)	No. of Aphids	Control (%)	No. of Aphids	Control (%)
Dusts:								
Lindane 75%	2	99+	0	100	134	83	207	78
Diazinon 25%	45	97	304	65	869	0	946	0
Trithion	72	90	111	87	697	10	912	4
Activated charcoal:								
Di-syston, 50%	6	99	6	99	90	88	15	98
Thimet 44%	1	99+	1	99+	105	86	310	67
Bayer 23129, 50%	10	99	60	93	326	58	339	64
Untreated	702	—	870	—	776	—	Plants killed[b]	

[a] Seed treated October 24, and planted November 26, 1956. Counts based on 120 seedlings.
[b] Control based on Diazinon plots.

Thimet was also included in the second planting. Aphid populations were moderately high and germination was good in all plots. Rapid plant growth in the October planting resulted in a short protection period. Results of these tests are summarized in table 3. At the 23-day count the Thimet plots showed only a 53% reduction in aphids. Cooler weather after the November planting gave much improved control. After 32 days both formulations of Thimet gave nearly complete control. After 45 days the Thimet emulsifiable concentrate plots showed some aphid damage whereas the technical Thimet plots were in good condition. The alfalfa in the other plots had been severely damaged or killed out.

COMPARISON OF THIMET AND DI-SYSTON APPLIED AT VARIOUS DOSAGES AND FORMULATIONS.—A comparison of spotted alfalfa aphid control was made with Thimet and Di-syston applied in technical form and as activated charcoal formulations at three dosages. Table 4 shows that all application rates gave about the same control after 28 days but the 48-ounce rate was usually most effective after 35 days. Additional counts were precluded by the appearance of large populations of predators, especially convergent lady beetles. Di-syston provided better control than Thimet, and the technical insecticides were more effective than charcoal formulations. There was no evidence of phytotoxicity in plants grown from treated seed, and there were no consistent growth differences between treated and untreated plots. Conversely, seed used in this test showed some phytotoxicity when tested under laboratory conditions.

COMPARISON OF DI-SYSTON IN TWO TYPES OF SOIL.—In further tests alfalfa seed was treated with Di-syston on activated charcoal at various dosages and planted on the same date in two types of soil, Superstition loamy sand, silted phase, and a much heavier Glendale silty clay. The mean temperature during the first 3 weeks was 64° F., providing for rapid plant growth. Results given in table 5 show that aphid populations increased rapidly in the Glendale clay. Predator activity on the loamy sand planting reduced populations after the 21-day counts. The 8-ounce dosage was adequate for the first 3 weeks, but control fell off after that. Slightly better control was obtained with the higher dosages. Apparently the type of soil in which the seed was planted made little difference in the aphid control. However, complicating predator activity in the loamy sand planting precluded definite conclusions.

COMPARISON OF THIMET AT DIFFERENT DOSAGES PLANTED ON TWO DATES.—Alfalfa seed was treated with different dosages of Thimet activated charcoal formulation on October 15, and, as in the previous tests, some was planted on that date and the rest on December 5. Mean temperatures for 3 weeks after the respective plantings were 64° and 54° F. Growth was rapid in the first planting but retarded by cooler weather in the second.

Table 3.—Control of the spotted alfalfa aphid with different insecticides applied to alfalfa seed as emulsifiable concentrates or technical insecticides.[a]

Treatment	October Planting				November Planting			
	After 23 Days		After 31 Days		After 25 Days		After 32 Days	
	No. of Aphids	Control (%)	No. of Aphids	Control (%)	No. of Aphids	Control (%)	No. of Aphids	Control (%)
Bayer 23129 (25% EC)	112	12	503	11	162	75	309	62
Bayer 23453 (technical)	133	—	350	38	172	73	336	57
Thimet:								
47.5% EC	47	53	189	66	15	98	30	96
Technical	—	—	—	—	1	98	7	99
6-Bromopiperonyl chrysanthemumate (technical)	142	—	574	—	528	18	711	9
Untreated	127	—	565	—	640	—	782	—

[a] Counts based on 60 seedlings.

Table 4.—Control of the spotted alfalfa aphid with different dosages of Thimet and Di-syston applied in activated-charcoal formulations and in technical form to alfalfa seed and effect of treatments on germination in laboratory.[a]

Insecticide	Ounces per 100 Lb. Seed	After 28 Days		After 35 Days		Germination (%)
		No. of Aphids	Control (%)	No. of Aphids	Control (%)	
Thimet:						
Activated charcoal	16	54	68	67	51	62
	32	36	78	165[b]	—	54
	48	56	66	96	30	52
Technical	16	56	66	129	7	78
	32	45	73	66	52	63
	48	16	90	42	70	52
Di-syston						
Activated charcoal	16	20	88	47	66	65
	32	18	89	82	41	64
	48	33	80	40	71	62
Technical	16	11	93	49	64	61
	32	26	84	24	83	58
	48	21	87	23	83	40
Untreated	—	166	—	138	—	83

[a] Counts based on aphids on 150 trifoliolate leaves.
[b] 125 on one leaf.

The 27-day count in the October planting was made on seedlings in the 5- to 6-leaf stage. As shown in table 6, aphid control was poor. The 26-day count in the December planting showed much better control when the plants were in the 2- to 3-leaf stage. The 36-day count in this planting indicated continued aphid control and little difference in control obtained by increased rates of Thimet. The reduction in the aphid population during the second count was due to convergent lady beetles.

LARGE-PLOT TEST OF THIMET AND DI-SYSTON AND THEIR EFFECT ON NODULATION.—This test was made on newly reclaimed desert land at Tacna, Arizona. Four adjacent 5-acre plots in a 30-acre field were planted with Chilean 21-5 alfalfa seed. The seed in three plots was treated with insecticide. In two of the treatments, one each with Thimet and Di-syston on activated charcoal, methylcellulose solution was used for a sticker at the rate of 3 quarts per 100 pounds of seed, and in the third, with Thimet containing 2% of dry methylcellulose, water was used at a 2-quart rate. The seed planted in the fourth plot was untreated. The seed was inoculated with nitrifying bacteria at recommended rates. The four plots were in a solid block, surrounded on the north, west, and south by alfalfa grown from untreated seed and on the east by open desert. The adjacent alfalfa on the north and west sides was used for additional checks. Because of an improper setting of the grain drill and extremely soft and irregular soil caused by rough leveling, many of the seeds were planted very deep. In many spots germination was poor or retarded. Cool weather also retarded plant growth and after 11 weeks many seedlings were only 2 to 3 inches high. Mean temperature was 59° F. for the first 3 weeks and 55° for 71 days after planting.

Table 6.—Control of the spotted alfalfa aphid with different dosages of Thimet on activated charcoal as affected by planting date and period between seed treatment and planting.

Ounces per 100 Lb. Seed	October Planting[a]		December Planting[b]			
	After 27 Days		After 26 Days		After 36 Days	
	No. of Aphids	Control (%)	No. of Aphids	Control (%)	No. of Aphids	Control (%)
8	403	9	274	36	45	58
16	329	26	112	74	39	63
24	338	24	79	81	41	61
32	332	25	101	76	47	56
64	332	25	53	88	34	68
Untreated	443	—	425	—	106	—

[a] Counts based on 120 trifoliate leaves.
[b] Counts based on 120 plants.

Beginning 21 days after planting, aphid counts were made at irregular intervals on 10 seedlings at each of 20 stops in each plot, including the south check plot, and also at each of 10 stops in the check strips of alfalfa on the north and west sides of the plots. Results given in table 7 show that all the treatments gave almost complete control for 71 days. A rapid buildup in the check plots required treatment on the 35th and 50th days. After 71 days the counts were discontinued because the check plots were replanted with treated seed. Before they were replanted, an aerial view from 700 feet did not show any difference in plant densities between treated and untreated plots.

After 78 days 100 seedlings 2 inches in height and selected at random from each plot were removed to a 6-inch depth with a $\frac{3}{4}$-inch soil tube, and the roots were washed

Table 5.—Control of the spotted alfalfa aphid on alfalfa seedlings grown from seed treated with Di-syston on activated charcoal and planted on two soil types.

Ounces per 100 Lb. Seed	On Superstition Loamy Sand				On Glendale Silty Clay					
	After Days Shown				After Days Shown					
	21[a]		35[b]		23[a]		35[b]		46[b]	
	No. of Aphids	Control (%)	No. of Aphids	Control (%)	No. of Aphids	Control (%)	No. of Aphids	Control (%)	No. of Aphids	Control (%)
8	47	70	63	27	18	89	160	64	1,329	48
16	16	90	42	51	22	87	76	82	655	74
24	33	79	19	78	25	85	159	63	1,045	59
32	6	96	13	85	15	91	270	38	644	75
64	19	88	15	82	9	94	201	54	437	83
Untreated	158	—	86	—	165	—	438	—	2,538	—

[a] Counts based on 120 plants.
[b] Counts based on 150 trifoliate leaves.

Table 7.—Control of the spotted alfalfa aphid and effect on nodulation in large-scale plantings of alfalfa from seed treated with Thimet and Di-syston on activated charcoal, with and without methylcellulose.

Test Plots	Aphids per 200 Seedlings After Day Shown						Nodules per 100 Seedlings
	21	26	32	39	48	71	
Untreated checks:							
South	35	15	201	61	551	84	620
North	—	—	38	53	89	59	—
West	—	—	186	11	—	—	—
Thimet (44%) plus							
Water	0	0	0	0	1	1	598
Methylcellulose	0	0	0	0	1	2	616
Di-syston (50%) plus							
Methylcellulose	2	0	0	0	0	1	568

and the nodules counted. The insecticides had little effect on nodulation.

References Cited

Davis, C. S., A. S. Deal, J. E. Dibble, R. C. Dickson, E. J. Dietrick, G. L. Finney, H. Graham, K. S. Hagen, I. M. Hall, Jr., J. K. Holloway, L. G. Jones, J. D. Paschke, B. Puttler, H. T. Reynolds, E. L. Schlinger, R. F. Smith, E. H. Stanford, V. M. Stern, E. S. Sylvester, and R. van den Bosch. 1957. The spotted alfalfa aphid and its control in California. Univ. California Agric. Ext. Serv., 44 pp.

Dickson, R. C., Edward F. Laird, Jr., and George R. Pesho. 1955. The spotted alfalfa aphid (yellow clover aphid on alfalfa). Hilgardia **24**(5): 93–118.

Dobson, R. C., and J. G. Watts. 1957. Spotted alfalfa aphid occurrence on seedling alfalfa as influenced by systemic insecticides and varieties. Jour. Econ. Ent. **50**(2): 132–5.

Koehler, C. S., and George G. Gyrisco. 1957. The systemic action of lindane in alfalfa upon the meadow spittlebug. Jour. Econ. Ent. **50**(3): 346–7.

Nielson, M. W. 1957. Sampling technique studies on the spotted alfalfa aphid. Jour. Econ. Ent. **50**(4): 385–9.

Nielson, M. W. 1958. Reaction of seedling alfalfa to the spotted alfalfa aphid in southern Arizona. Jour. Econ. Ent. **51**(5): 601–3.

Reynolds, H. T., and L. D. Anderson. 1955. Control of the spotted alfalfa aphid on alfalfa in southern California. Jour. Econ. Ent. **48**(6): 671–5.

Reynolds, H. T., T. R. Fukuto, R. L. Metcalf, and R. B. March. 1957. Seed treatment of field crops with systemic insecticides. Jour. Econ. Ent. **50**(5): 527–9.

Tuttle, D. M., and G. D. Butler, Jr. 1954. The yellow clover aphid—a new alfalfa pest in the Southwest. Jour Econ. Ent. **47**(6): 1157.

Effect of Root Size and Soil Moisture on the Number of Clover Root Borers Present in Red Clover Roots[1]

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Abstract

During 2 consecutive years, similar field experiments were conducted in New York to study the effects of red clover (*Trifolium pratense* L.) root size and percentage available soil moisture on numbers of the clover root borer, *Hylastinus obscurus* (Marsh.) (Scolytidae: Coleoptera).

In 1956, as soil moisture decreased, borer numbers increased; as root size increased, borer numbers increased. A multiple regression analysis indicated that either root size or soil moisture could account for most of the variation in borer numbers, because the two independent variables were closely correlated to each other; as soil moisture decreased, root size increased. In 1957, it was found that root size alone was responsible for the majority of the variation in borer numbers.

On the basis of these data and differences in the number of areas sampled, the distance between sampling areas, stand uniformity, and ranges of soil moisture in 1956 as compared with 1957, the conclusion was reached that root size has a more direct influence than soil moisture on borer numbers. Soil moisture is believed to indirectly affect borer numbers owing to its influence on root size.

The clover root borer, *Hylastinus obscurus* (Marsh.), feeds and reproduces mainly in the tap roots of red clover, (*Trifolium pratense* L.) in which both the larvae and adults form characteristic sinuous galleries. The adults, after overwintering in roots in which the immature stages developed, migrate largely by flight during the spring. Infestation of other plants in the same or distant fields is accomplished in this manner.

During the conduct of chemical control and biological experiments in New York over a period of 20 years, it has been observed that, on the average, more borers are present in the larger than in the smaller roots. Newsom (1948) demonstrated this relationship in both first- and second-harvest year roots.

Several workers have indicated that borers cause greater root damage where soil moisture is low than where sufficient soil moisture is available (Webster 1905, Folsom 1909, Rockwood 1926). In laboratory experiments carried on in Ohio, Pruess (1957, Pruess & Weaver 1958), however, found that damage, as measured by reduction in foliage yield, increased only slightly as soil moisture decreased, but they found also that a greater number of borers was present under the drier conditions. They suggested that the response of borers to moisture may be ascribable to the attractiveness of low-moisture conditions to adult borers, greater oviposition, or increased larval survival.

On the basis of these data, investigation of the effect of

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both soil moisture and root size on the number of borers present in field-collected roots seemed worthwhile.

EXPERIMENTAL PROCEDURE.—In early June 1956, six gypsum soil moisture blocks were buried at a depth of 4 inches in the soil of a red clover field. All blocks were situated about 50 feet apart on a hillside, and the position for each block (referred to hereafter as moisture station) was selected in a restricted randomized manner in an attempt to obtain a wide range of soil moistures.

Long wire leads extending from each block were severed at a height of 5 inches above the soil surface. Prior to hay harvests, the shortened leads were forced beneath the plant debris so that uninterrupted mowing of the field could be accomplished by the grower. After harvests, the moisture stations were relocated by triangulation.

From June to September 1956, 10 readings of resistance in ohms, which were later converted to percentage available soil moisture, were taken at each station using a Bouyoucos soil moisture bridge. In December of that year, 10 plants were dug from the immediate vicinity of each station. After cutting through each root at the crown, the average crown diameter in millimeters was computed for use as an index of root size. Roots were then dissected under magnification to locate the borers present.

A similar experiment was conducted during 1957 in the same county. Fifteen soil moisture blocks were buried at wide intervals in a 5-acre red clover field, and 23 readings of ohms resistance were taken during the period extending from April to October. A total of 41 roots was taken from each station on 12 different dates irregularly spaced between May and October, and examined for root borers in the manner previously described.

A statistical analysis of each year's data was conducted separately, using multiple regression. In this way the two independent variables—soil moisture and root size—could be examined together in regard to their association with borer numbers.

Table 1.—Numbers of clover root borers found in red clover roots taken from first harvest year fields in which readings of percentage available soil moisture had been recorded. Oswego County, New York.

YEAR	STATION	SEASONAL AVERAGE PERCENTAGE ASM[a]	AVERAGE CROWN DIAMETER (MM.)	TOTAL NO. OF BORERS FOUND
1956	1	79	10.7	7
	2	83	12.2	9
	3	69	12.7	27
	4	79	13.0	16
	5	54	14.2	28
	6	53	14.5	34
1957	A	64	8.5	65
	B	79	8.5	79
	C	77	8.8	68
	D	80	8.8	98
	E	65	8.9	97
	F	75	9.3	127
	G	76	9.3	150
	H	78	9.4	119
	J	66	9.4	139
	K	76	9.5	63
	L	70	9.6	99
	M	67	9.7	154
	N	71	9.8	79
	O	87	9.8	129
	P	76	10.1	166

[a] Available soil moisture.

Table 2.—Analysis of component sources of variation associated with the multiple regression of root size and soil moisture on root borer numbers.

SOURCE OF VARIATION	1956 Standard Partial Regression Coefficient	1956 Significance at 5% Level	1957 Standard Partial Regression Coefficient	1957 Significance at 5% Level
Root size and moisture	—	*	—	n.s.
Root size after moisture	0.3828	n.s.	1.0002	*
Moisture after root size	0.5491	n.s.	0.0097	n.s.

RESULTS AND DISCUSSION.—Although most of the borers found were adults, some larvae and pupae were taken. The different stages were pooled, as shown in table 1, where data on root size and percentage available soil moisture are also presented.

For the 1956 data, a significant multiple regression was detected (table 2), with 88% of the variation in borer numbers accounted for by variations in root size and soil moisture. As percentage available soil moisture decreased, numbers of borers increased (table 3). When root size increased, borer numbers also increased. In addition, the two independent variables were found to be closely correlated with each other, with crown diameter increasing as percentage available soil moisture decreased.

Examination of the standard partial regression coefficients (table 2) will show that neither root size nor soil moisture, when considered after the effect of this one on the other has been discounted, could account for the variations in borer numbers. This situation arises because of the close correlation between root size and soil moisture. Either of the independent variables alone, therefore, accounts for the majority of the variation in numbers of borers, and the small amount of additional variation that the other independent variable explains is unimportant.

Analysis of the 1957 data did not disclose a significant multiple regression (table 2). Variations in root size and soil moisture together accounted for only 35% of the variation in numbers of borers. Reference to the standard partial regression coefficient, however, indicates that root size, after the effect caused by soil moisture had been subtracted, accounts for a significant portion of the variation in borer numbers.

The discrepancies between the 1956 and 1957 data are readily apparent. These differences, however, were considered to be of value in elucidating a plausible explanation of the relationship of numbers of borers to soil moisture and root size.

Table 3.—Correlation coefficients showing relationships among soil moisture, root size, and clover root borer numbers.

RELATIONSHIP	SIMPLE CORRELATION COEFFICIENTS 1956	1957
Soil moisture: borer numbers	−0.9112[a]	0.0660
Root size: borer numbers	0.8747[a]	0.5924[a]
Soil moisture: root size	−0.8266[a]	0.1274

[a] Significant at the 5% level.

In 1956, only six stations were under consideration. These were located much closer together than were the 15 stations used in 1957. Furthermore, stand density was far more uniform in the field used in 1956 as compared with 1957, even though both fields were in the first harvest year. It would seem that borers migrating into fields having nonuniform stands, would tend to infest a greater percentage of plants, where only a few plants were available, than where many were present. It is thought this fact, was responsible for the great amount of unexplained variation among the 1957 data.

The wider range of soil moisture encountered in 1956 could account in part for the close correlation of borer numbers with soil moisture found in 1956 and the absence of a similar correlation in 1957. This condition would still permit the existence of a close correlation between borer numbers and root size during both years.

The short distance between stations was probably associated in part with the significant correlation found between soil moisture and root size in 1956. In 1957, with stations widely dispersed, this relationship could not be detected, possibly because of fertility differences in that field.

The fact that a close correlation between borer numbers and root size was detected during both years supports the contention that root size has a more direct influence than soil moisture on borer numbers. It is believed that soil moisture indirectly affects borer numbers, owing to its influence on root size.

Reasons for the increased number of borers found in the larger roots have not been fully investigated. Although a definite trend toward greater oviposition has been demonstrated when crowding was reduced, the probablity that borers in the field encounter a large root sooner than a small one is considered a more reasonable explanation.

REFERENCES CITED

Folsom, J. W. 1909. The insect pests of clover and alfalfa. Illinois Agric. Expt. Sta. Bull. **134.**

Newsom, L. D. 1948. The biology and economic importance of the clover root borer, *Hylastinus obscurus* (Marsham). Thesis for the degree of Ph.D. Cornell University, Ithaca, New York.

Pruess, K. P. 1957. Studies on the clover root borer, *Hylastinus obscurus* (Marsham). Thesis for the degree of Ph.D. The Ohio State University, Columbus, Ohio.

Pruess, K. P., and C. R. Weaver. 1958. Estimation of red clover losses caused by the clover root borer. Jour. Econ. Ent. **51**(4): 491–2.

Rockwood, L. P. 1926. The clover root borer. U. S. Dept. Agric. Dept. Bull. **1426.**

Webster, F. M. 1905. The clover root-borer. U. S. Dept. Agric. Ent. Circ. **67.**

Synergistic Action of Piperonyl Butoxide with Bayer 21/199 and Its Corresponding Phosphate in Mice[1]

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ABSTRACT

The joint oral administration of piperonyl butoxide (1:5) increased the toxicity of both Bayer 21/199 (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) and its corresponding phosphate to mice four- to six-fold. This increase in toxicity was also found when synergist and toxicant were administered by different routes. Piperonyl butoxide increased the *in vivo* but not the *in vitro* inhibition of mouse brain cholinesterase by 21/199 or its phosphate. Preliminary studies with P^{32}-labeled 21/199 demonstrated that the joint administration of piperonyl butoxide inhibited its metabolism to more polar metabolites.

Several of the pyrethrum synergists have been found to increase the toxicity of certain organic phosphates to body lice (Eddy *et al.* 1954) and to house flies (*Musca domestica* L.) (Hoffman *et al.* 1954). The compounds that showed the greatest degree of synergism were the *O*-(3-chloro-4-methylumbelliferone) or *O*-(4-methylumbelliferone) esters of *O,O*-dialkyl phosphorothioate. Bayer 21/199 (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) is among this group. Recently this compound was shown to be an effective chemotherapeutic agent for migrating cattle grubs when applied as a spray (Brundrett *et al.* 1957). These data suggested the use of a synergist to increase the systemic effectiveness of this insecticide against cattle grubs. However, in a chemotherapy experiment conducted by associates at the Corvallis, Oregon, laboratory, the toxicity of Bayer 21/199 to cattle appeared to have been increased by the joint oral administration of piperonyl butoxide (Eddy & Roth, unpublished data).

As far as is known, no other information is available on the toxicity of such combinations to mammals. Studies on mice were undertaken to determine whether organophosphorus synergism does occur in mammals and if so, to study its mode of action. Since many organic phosphorothioates undergo *in vivo* oxidation to their corresponding phosphates, which are usually more potent anticholinesterase agents (Casida 1956), the phosphate of 21/199 (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphate) was also studied.

MATERIALS AND METHODS.—Adult white mice were employed in these studies. The compounds[3] were administered as polyethylene glycol 400 solutions with a

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[3] The Bayer 21/199 and its corresponding phosphate were purified samples supplied by Geary Research Laboratory, New York. The piperonyl butoxide was an analytical standard from the Fairfield Chemical Co., Baltimore, Md

0.25 cc. tuberculin syringe. The concentration was adjusted so that the volume administered was 0.4% of the body weight of the mouse. For acute oral toxicity tests a minimum of 10 mice were used at each dosage and the animals were observed for 7 days after treatment.

The *in vitro* inhibition of cholinesterase (ChE) was determined by incubating 1.7 ml. of mouse brain brei containing 12.5 mg. of tissue in 0.134 M phosphate buffer, pH 7.2, with 0.1 ml. acetone solution of the inhibitor for 30 minutes at 37° C., adding 0.2 ml. of 0.025 M acetycholine chloride, and incubating for 5 minutes at the same temperature. The remaining acetylcholine (ACh) was determined colorimetrically (Hestrin 1949).

For *in vivo* studies 1.8 ml. of mouse brain brei (25 mg. of tissue) in 0.134 M phosphate buffer was incubated with 0.2 ml. of 0.05 M ACh (final concentration 5×10^{-3} M) for 15 minutes at 37° C. The remaining ACh was determined by the colorimetric method.

The P^{32}-21/199[4] employed in these studies had an observable specific activity of 6.29×10^{8} cpm/gram and a radiochemical purity of 98% as determined by paper chromatography. The radiometric assay was performed with a conventional scaler and a windowless gas-flow proportional counter. All samples were counted in duplicate for a sufficient time to attain a standard error of ±5%. The mean counting rate was corrected for background, self absorption, and decay, and converted to microgram-equivalents of 21/199.

Total radioactive compounds present in tissues were determined by assay of planted samples of aqueous homogenates of the tissue containing 25 to 100 mg. of tissue per milliliter of homogenate. The chloroform-soluble compounds were determined by adding 0.2 ml. of 50% trichloroacetic acid solution to 5 ml. of the homogenate in a stoppered centifuge tube. Five milliliters of chloroform were added to the tube and the contents thoroughly shaken. Following centrifugation to insure separation of the two layers, 1- to 2-ml. samples of the chloroform layer were assayed radiometically. When P^{32}-labeled 21/199 or its phosphate was added to control tissue homogenates and treated in this manner, 99.1% of the 21/199 and 90.4% of the phosphate (means of 3 determinations) were found to partition into the chloroform. The systems employed for paper chromatographic analysis have been previously reported (Robbins *et al.*, 1959).

Table 1.—Acute oral toxicity of Bayer 21/199 and its corresponding phosphate (0-21/199) to mice, alone and in combination with piperonyl butoxide (PB) at a 1:5 ratio.

	Per Cent Mortality				
	21/199		0-21/199		
Dosage (mg./kg.)	Alone	Plus PB	Alone	Plus PB	PB
1.6		0		0	
3.1		10		13	
6.3		40		40	
12.5	0	100	0	100	
25.0	33		20		
50.0	70		92		
75.0	90		100		
100.0	100				
500.0					0
Total number of animals	52	42	58	55	10

Table 2.—Toxicity of Bayer 21/199 with and without piperonyl butoxide (PB) following administration by different routes, 6 mice per treatment.

Dosage	Route of Administration	Per Cent Mortality
Piperonyl butoxide[a] (62.5 mg./kg.)	Intraperitoneal	0
21/199[a] (12.5 mg./kg.)	Oral	0
21/199 plus PB (12.5 and 62.5 mg./kg.)	Oral and intraperitoneal	100

[a] Equal volume of the solvent, polyethylene glycol 400, administered by the alternate route.

Studies on the *in vitro* conversion of 21/199 to a more potent anti-ChE agent, by mouse liver homogenates, were conducted, employing the method described by Davison (1955). For the activation of 21/199, 1.8 ml. of 25% liver-brei supernatant, in 0.15 M sodium chloride, 0.015 M magnesium sulfate, and 0.008 M nicotinamide were incubated for 15 minutes at 37° C. with 0.1 ml. of diphosphopyridine nucleotide (DPN) solution (8 mg./ml) and 0.1 ml. of acetone solution of 21/199 (final concentration 1×10^{-4} M). The ChE-inhibitor(s) formed were assayed by incubating 0.1 ml. of the reaction mixture with mouse brain ChE (25 mg. of tissue) for 30 minutes at 37° C. ACh was then added (final concentration 5×10^{-3} M), and incubated for an additional 15 minutes at 37° C. and the remaining ACh determined colorimetrically. The anti-ChE agents produced in the *in vitro* system were detected on paper chromatograms with the spot test described by Cook (1955).

RESULTS.—*Toxicity.*—The acute oral LD-50 for both 21/199 and its phosphate was between 25 and 50 mg./kg. (table 1). The phosphate was somewhat more toxic, giving 92% mortality at 50 mg./kg. as compared with 70% for 21/199. The acute oral toxicity of both compounds was increased four to six times by the joint administration of piperonyl butoxide.

To determine if the increase in toxicity of 21/199 plus piperonyl butoxide might be the restult of enhanced absorption of the toxicant from the digestive tract, owing to the presence of the piperonyl butoxide, 21/199 was administered orally and the piperonyl butoxide was injected intraperitoneally. The results in table 2 demonstrated that the toxicity of 21/199 was increased by piperonyl butoxide when the two compounds were administered jointly by different routes.

Anti-ChE Activity.—Results of *in vitro* determinations demonstrated that the phosphate of 21/199 was several thousand times more potent as a anti-ChE agent for mouse brain ChE than 21/199 (table 3). When piperonyl butoxide was added with the toxicants to the *in vitro* system, no significant increase in anti-ChE activity was detected. Piperonyl butoxide alone was ineffective as a ChE inhibitor at the concentrations employed in the *in vitro* studies.

[4] Synthesized by Farbenfabriken Bayer, Germany.

Table 3.—Per cent *in vitro* inhibition of mouse brain ChE by Bayer 21/199 and its corresponding phosphate (0–21/199), alone and in combination with piperonyl butoxide (PB) at a 1:5 ratio.

Molar Concentration	Per Cent Inhibition	
	Inhibitor	Inhibitor Plus PB
	21/199	
5×10^{-3}	53	40
1×10^{-3}	37	28
5×10^{-4}	33	23
1×10^{-4}	27	16
	0–21/199	
2.5×10^{-6}	96	98
1×10^{-6}	60	63
5×10^{-7}	40	41
1×10^{-7}	13	12

Piperonyl butoxide increased the *in vivo* anti-ChE activity of 21/199 and its phosphate (table 4). When piperonyl butoxide was administered alone at the same dosage rate no *in vivo* inhibition of mouse brain ChE was detected.

Studies with P^{32}-labeled 21/199.—The data from the distribution studies with radioactive 21/199 (table 5) indicate that the joint administration of piperonyl butoxide prevented the enzymic hydrolysis of 21/199 to more polar degradation products.

Of the tissues studied, excluding the digestive system, only the liver of the mice treated with 21/199 plus piperonyl butoxide contained sufficient organosoluble radioactive compounds to permit chromatographic analyses. Two compounds were detected, one that ran with the Rf of the parent compound and another that behaved chromatographically like the phosphate of 21/199.

In vitro Activation of 21/199.—The conversion of 21/199 by enzymic oxidation to its corresponding phosphate was tested employing mouse liver brei in the *in vitro* system described by Davison (1955). The results of enzymic assays (table 6) indicate that 21/199 was converted to a more potent anti-ChE agent by this system. Aliquots of the reaction mixture containing the unknown inhibitor were extracted with an equal volume of chloroform. Enzymic assay of the aqueous fraction following extraction demonstrated that the anti-ChE agent had partitioned into the chloroform layer. When chloroform extracts were concentrated and analyzed chromatographically and the chromatograms assayed for anti-ChE agents according to the method of Cook (1955), using horse-serum ChE, a compound that ran with the Rf of the phosphate of 21/199 was detected.

Table 4.—*In vivo* inhibition of mouse brain ChE following administration of Bayer 21/199 and its corresponding phosphate (0–21/199) at 12.5 mg./kg. alone and in combination with piperonyl butoxide (PB) at 62.5 mg./kg.

Treatment	½ Hour		1 Hour	
	Micromoles[a] ACh Hydrolyzed	Per Cent[b] Control Activity	Micromoles[a] ACh Hydrolyzed	Per Cent[b] Control Activity
Piperonyl butoxide:				
Alone	4.08	100	3.98	98
21/199:				
Alone	3.61	89	3.42	84
Plus PB	2.81	69	0.31[c]	8
0-21/199:				
Alone	3.18	78	3.01	74
Plus PB	0.29[d]	7	—	—

[a] Average values for 2 to 4 mice.
[b] Control ChE activity based on the mean of 16 male mice.
[c] Both mice severely affected, maximum survival time for this dosage is about 1½ hours.
[d] Average for 4 mice, 2 severely affected and 2 without symptoms, range 0–0.61 micromoles. Maximum survival time about 40 minutes.

Table 5.—Distribution of radioactive compounds in tissues and urine of male mice 1 hour after oral administration of 12.5 mg./kg. of P^{32}-Bayer 21/199 alone and with piperonyl butoxide (PB).

Tissue	Microgram-Equivalents of 21/199 per Gram Tissue			
	21/199[a]		21/199+PB (1:5)[a]	
	Total	Organo-soluble	Total	Organo-soluble
Blood[b]	1.3	0	2.0	0.2
Brain	0	0	1.1	.6
Lungs	.8	0	3.5	.8
Spleen	.7	0	1.3	.4
Testes	0	0	.9	.3
Heart	0	0	2.5	1.1
Muscle	.4	0	1.1	.5
Liver	8.4	.9	17.0	3.9
Kidney	4.5	.6	3.6	1.6
Stomach	685.9	605.8	595.1	520.8
Small intestine	9.4	5.0	18.0	13.4
Cecum	1.2	0	1.9	.7
Large intestine	.6	0	1.7	.6
Fat	—	.3	—	.8
Urine[b]	104.0	—	21.4	—

[a] Average of 2 mice.
[b] Microgram equivalents per milligram.

Discussion.—The phosphate of 21/199 was approximately equitoxic to 21/199 by oral administration, alone or synergized, even though it was found to be several thousand times as potent an anti-ChE agent against mouse brain ChE *in vitro*. These differences are probably due in part to a decrease in stability of the phosphate to biochemical hydrolysis. The same was found for topically applied 21/199 and its phosphate in the house fly (Monroe & Robbins 1959). The increase in the *in vivo* but not in the *in vitro* anti-ChE activity produced by piperonyl butoxide with both compounds and the lack of either *in vivo* or *in vitro* anti-ChE activity of the synergist indicate that its mode of action differs from that of the toxicants. This was further substantiated by the metabolism studies

Table 6.—Activation of Bayer 21/199 by mouse liver brei.

	Micromoles ACh Hydrolyzed	Per Cent Inhibition
Control	3.86	
Liver brei, 21/199	3.62	6
Liver brei, DPN, 21/199	0.70	82

with P^{32}21/199, in which the joint application of the synergist resulted in a more widespread distribution and higher concentrations of labeled organosoluble compounds in the tissues. These data indicate that the synergist may increase toxicity by blocking the metabolism of the toxicant. This has been postulated as the mode of action of piperonyl butoxide in pyrethrum synergism (Chamberlain 1950).

The formation *in vivo* and *in vitro* of a more potent anti-ChE agent from 21/199, which behaved chromatographically like the phosphate, suggests that this phosphorothioate exerts its toxic action following *in vivo* conversion to its phosphate.

REFERENCES CITED

Brundrett, H. M., W. S. McGregor, and R. C. Bushland. 1957. Systemic cattle grub control with Bayer 21/199 sprays. Agric. Chemicals **12:** 36–7, 123–5.

Casida, John E. 1956. Metabolism of organophosphorus insecticides in relation to their antiesterase activity, stability and residual properties. Jour. Agric. and Food Chem. **4:** 772–85.

Chamberlain, Roy W. 1950. An investigation on the action of piperonyl butoxide and pyrethrum. Amer. Jour. Hyg. **5:** 153–83.

Cook, J. W. 1955. Paper chromatography of some organic phosphate insecticides. IV. Spot test for *in vitro* cholinesterase inhibitors. Jour. Assoc. Off. Agric. Chem. **38:** 150–3.

Davison, A. N. 1955. The conversion of schradan (OMPA) and parathion into inhibitors of cholinesterase by mammalian liver. Biochem. Jour. **61:** 203–9.

Eddy, Gaines W., M. W. Cole, and A. M. Marulli. 1954. Louse powder synergists. Soap and Chem. Spec. **30:** 121–3, 143.

Hestrin, S. 1949. The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine and its analytical application. Jour. Biol. Chem. **180:** 249–61.

Hoffman, Robert A., Theodore L. Hopkins, and Arthur W. Lindquist. 1954. Tests with pyrethrum synergists combined with some organic phosphorus compounds against DDT-resistant flies. Jour. Econ. Ent. **47:** 72–6.

Monroe, Ronald E., and William E. Robbins. 1959. Studies on the mode of action of synergized Bayer 21/199 and its corresponding phosphate in the house fly. Jour. Econ. Ent. **52:** 643–7.

Robbins, William E., Theodore L. Hopkins, Darrell I. Darrow, and Gaines W. Eddy. 1959. Studies with P^{32}-Bayer 21/199 sprayed on cattle. Jour. Econ. Ent. **52**(2): 214–7.

Studies on the Control of Alfalfa Weevil Larvae in the East[1]

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ABSTRACT

Of 27 insecticides tested in Maryland for control of larvae of the alfalfa weevil (*Hypera postica* (Gyll.)), aldrin, dieldrin, endrin, heptachlor, and Thiodan® (6,7,8,9,10,10-hexachloro-1,5*a*, 6,9,9*a*-hexahydro-6,9,-methano-2,4,3-benzodioxathiepin-3-oxide) were the most effective. Sprays containing 4 ounces of heptachlor per acre applied in late April provided better control than sprays applied earlier or later. Little difference in control was obtained when 5, 10, or 20 gallons of water were used. Granular insecticides alone or mixed with fertilizer were less satisfactory and required heavier dosages. Mixtures were generally more effective than a single insecticide to control simultaneously the alfalfa weevil, pea aphid (*Macrosiphum pisi* (Harr.)), and meadow spittlebug (*Philaenus leucopthalmus* (L.)).

The alfalfa weevil, *Hypera postica* (Gyll.), is now an important pest in the East. Since it was first found in Maryland in 1952, it has spread into 13 Eastern States from Georgia to Massachusetts. In most areas severe damage to alfalfa occurred the second year after the weevil was found. In areas that have been infested for several years there has been an almost total loss of first-crop alfalfa in untreated fields every year. Damage is restricted largely to the first crop, since most of the larvae mature by the time it is harvested. Some larvae are present on the second crop, but the amount of feeding and damage varies with the location and season. If large numbers of newly emerged adults or larvae are present when the first crop is cut, they will feed on the new shoots and delay the growth of the second crop.

From 1953 to 1957 investigations were conducted in Maryland to control larvae of this weevil with insecticides. The 1953 studies showed that the early-spring treatment used in the West was not effective in the East, especially against larvae hatching 2 to 3 weeks after application (App 1954). This fact may be explained in part by certain differences in the biology of the weevil in the East, as noted by Manglitz & App (1957). The insecticide studies reported here were designed (1) to find effective insecticides; (2) to determine the minimum effective dosages, the optimum time of application, and the number of gallons of water to use per acre; (3) to evaluate the effectiveness of granulated insecticides alone and with fertilizers; and (4) to find an insecticide or mixture that would also provide control of the pea aphid (*Macrosiphum pisi* (Harr.)) and meadow spittlebug (*Philaenus leucophthalmus* (L.)).

MATERIALS AND METHODS.—Most of the insecticides were applied as emulsion sprays with power equipment calibrated to deliver approximately 10 gallons per acre at 100 p.s.i. The treatments were usually made on plots 43×200 feet and replicated four times. In a few experiments plots 20×20 or 30×30 feet were treated with hand equipment. The effect of the treatments was determined by taking samples of the weevil population with a 15-inch insect net. In some of the experiments effects of the treatments on plant height and yield were obtained by measur-

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[2] G. R. Manglitz and R. B. Knapp (deceased) assisted in the conduct of these studies.

Table 1.—Effect of different insecticides on the alfalfa weevil larval population, 1953–57.

Insecticide (in Emulsion Unless Otherwise Indicated)	Ounces per Acre	Year Tested	Per Cent Reduction After— 1–2 Weeks	2–3 Weeks	3–4 Weeks
Large-plot tests					
Aldrin	4	1955	99+	88	19
Diazinon®[a]	24	1954	90	38	—
Dieldrin	4	1953	—	100	63
Heptachlor	4	1954	—	99	67
Lindane	8	1954	96	46	—
Malathion	16	1955	80	—	7
Methoxychlor	24	1953	—	100	47
Phosdrin	8	1956	73	48	4
Perthane®[b]	24	1955	90	—	47
Strobane®	32	1954	91	5	—
Toxaphene[c]	24	1953	—	75	29
Small-plot tests					
Di-syston®[d]	8	1956	58	22	—
Chlorthion®[e]	8	1956	64	33	—
DDVP	16	1955	79	60	5
Demeton	8	1956	56	—[m]	—
Dicapthon	8	1957	93	27	—
Dilan®[f]	16	1954	87	48	—
Endrin	5	1957	98	91	94
Ethion (suspension)	8	1956	63	36	—
Guthion®[g]	16	1956	87	82	—
Phostex®[h]	16	1956	54	3	—
Rotenone (dust)	8	1955	63	28	—[m]
Sevin®[i]	8	1956	20	1	—
Tephrosia[j]	8	1955	41	11	8
Thimet®[k]	16	1956	86	44	—
Thiodan	8	1957	98	75	—
Trithion®[l]	8	1956	57	38	—

[a] *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.
[b] 1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane.
[c] A mixture of chlorinated terpenes with about 66% chlorine.
[d] *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate.
[e] *O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate.
[f] 1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts 1,1-bis(*p*-chlorophenyl)-2-nitrobutane.
[g] *O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate.
[h] A mixture of bis(dialkyloxyphosphinothioyl) disulfides.
[i] 1-naphthyl-*N*-methyl carbamate.
[j] Emulsion containing 3 grams of rotenone per 100 ml. Prepared from an extract of *T. vogelii*.
[k] *O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate.
[l] *S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate.
[m] Population higher than the check.

ing 10 plants and cutting and weighing a forage sample 34 inches×20 feet in each plot.

Effectiveness of Various Insecticides.—A total of 27 insecticides were tested for control of alfalfa weevil larvae. Population samples were taken approximately 1 to 2, 2 to 3, and 3 to 4 weeks after application. The data from the different experiments are summarized in table 1. The results given are from experiments covering several years, but the weevil infestations and the methods of application and obtaining data are sufficiently similar to allow comparisons. The most promising insecticides were aldrin, dieldrin, endrin, heptachlor, and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3,-benzodioxathiepin-3-oxide).

Minimum Effective Dosages.—Experiments were conducted with the more effective materials to determine the minimum dosages that would provide effective control. The data, summarized in table 2, show that all the

Table 2.—Effect of different dosages of insecticides on the population of alfalfa weevil larvae, 1955 and 1957.

Insecticide	Ounces per Acre	Per Cent Reduction After— 1–2 Weeks	2–3 Weeks	3–4 Weeks
Large-plot tests, 1955				
Aldrin	2	97.8	79.9	18.9
	4	99.8	88.5	18.9
Dieldrin	1	98.1	77.0	15.7
	2	99.5	87.5	36.2
Endrin	2	93.4	71.3	—[a]
	6	98.5	84.9	—[a]
	8	99.8	86.8	—[a]
Heptachlor	1	99.5	72.9	—[a]
	2	99.5	77.8	—[a]
Small-plot tests 1957				
Phosdrin	4	100.0	18.8	—
	8	95.1	42.8	—
Thiodan	8	98.4	75.0	—
	16	96.7	61.6	—

[a] Population higher than the check.

materials provided good initial control, but in general the heavier dosages were more effective after 2 to 3 weeks. The effectiveness of all dosages of all materials decreased rapidly after 3 weeks.

Optimum Time of Application.—Heptachlor at 4 ounces per acre was applied on several dates in 1955, 1956, and 1957, to determine the effect of the time of application on the control obtained. In 1956 and 1957 data were also obtained on the advantages of two applications of heptachlor to the same plots.

Table 3 gives the data obtained in 1955. The first two applications were not effective because they were too early to affect the peak larval hatch. Applications made on April 18, when the plants were 9 inches tall, showed good control when sampled on April 27 and May 6, but not on May 19. However, the spraying protected the crop until harvest, and a good crop was obtained. Spraying on May 2 provided good control of weevil larvae until harvest, but considerable damage had occurred previous to the treatment.

In 1956 data were also taken on the percentage of tips

Table 3.—Effect of date of application on control of alfalfa weevil larvae with heptachlor at 4 ounces per acre, 1955–57.

Date Treated	Height of Plants (in.)	Per Cent of Tips Infested	Per Cent Reduction		
1955			*April 27*	*May 6*	*May 19*
March 14	2.3	—	16	38	58
April 1	3.1	—	51	10	28
18	9.4	—	99+	99	20
May 2	14.6	—	—	99+	91
1956			*May 10*	*May 16*	*May 24*
April 5	2.7	0	38	72	70
18	6.0	10	88	74	66
30	10.0	80	99	96	72
May 10	15.4	100	—	100	99
April 5 and 18	—	—	96	90	98
18 and 30	—	—	100	97	91
30 and May 10	—	—	99	100	100
1957			*April 26*	*May 3*	*May 16*
April 15	5.8	63	99+	40	93
30	18.4	100	—	98	92
15 and 30	—	—	99+	100	100

Table 4.—Effect of date of application of heptachlor at 4 ounces per acre on height and yield of alfalfa plants, 1956–57.

Date Treated	Height of Plants (in.)	Green Weight Yield (tons/acre)	Per Cent Increase in	
			Height	Yield
1956	*May 18*			
Untreated	18.1	2.5	—	—
April 5	20.6	3.7	14	48
18	21.7	4.0	20	60
30	23.0	4.8	27	92
May 10	20.6	3.6	14	44
April 5 and 18	22.4	4.1	24	64
18 and 30	21.6	3.6	19	44
30 and May 10	22.8	4.9	26	96
1957	*May 21*			
Untreated	22.9	3.0	—	—
April 15	29.4	5.4	28	80
30	26.4	4.2	15	40
April 15 and 30	28.3	4.3	24	43

showing infestation at the time of treatment (table 3). Applications on April 5 were too early for good control and those on May 10 too late to prevent damage. The best control was obtained from the April 30 treatment, when the plants averaged 10 inches tall and 80% of the tips were infested. Little benefit resulted from two applications to the same plots. Of the plots receiving one application, those treated on April 30 showed the greatest increase in plant height and yield (table 4). No marked increase in height or yield resulted from two applications.

In 1957 additional data were obtained on plots treated on April 15 or April 30 and on plots treated on both dates (table 3). All the treatments gave good initial control. By May 3 the April 15 treatment had decreased in effectiveness, but on May 16 equally good results were obtained from all application dates. The 1957 season was unusually early and many larvae completed development before May 16, which fact accounts for the high controls obtained. All the treatments resulted in an increase in plant height and yield (table 4).

Effect of Differing Amounts of Water.—To determine the effects of applying the insecticide in differing amounts of water per acre, alfalfa plots were treated with 4 ounces of heptachlor in 5, 10, and 20 gallons of water per acre. Table 5 shows that equally good control was obtained regardless of the amount of water used.

Effectiveness of Granulated Insecticides.— Several workers, including Muka (1957), Blackburn (1957), and Walstrom & Lofgren (1957), have reported good control of weevil larvae with granulated insecticides applied in the fall or early spring. In 1957 three granulated insecticides formulated on 30 to 60 attapulgite were applied on March 20 to 30×30 foot plots of alfalfa. Two dosages were used, and each treatment was replicated four times.

Table 5.—Effect on alfalfa weevil larvae of spraying 4 ounces of heptachlor in different amounts of water per acre, 1956.

Gallons of Water per Acre	Number of Larvae per 100 Sweeps		Per Cent Reduction	
	May 11	May 21	May 11	May 21
Untreated	422	5759	—	
5	0	501	100	91
10	6	578	99	90
20	1	467	99+	92

Table 6.—Effect of treating alfalfa with granulated insecticides for control of alfalfa weevil larvae, 1957.

Insecticide[a]	Ounces per Acre	Per Cent Reduction		
		April 22	May 2	May 15
Heptachlor	8	92	58	95
	16	99+	77	98
Endrin	8	38	0	0
	16	50	0	31
Dieldrin	8	77	0	94
	16	90	53	96

[a] Granules applied on March 20.

Table 6 shows that on April 22 heptachlor was more effective than dieldrin at both dosages and endrin was not effective at either dosage. The good control shown on May 15 is due to the early pupation in 1957.

Insecticide-Fertilizer Mixtures.—On March 15, 1957, about 2 acres of a 6-acre alfalfa field were treated with 1 pound of granulated heptachlor mixed with 500 pounds of 0–10–20 fertilizer per acre; the remainder of the field received only the fertilizer. Counts taken on April 22 and April 29 showed 63 and 54% fewer larvae, respectively, in the treated area. However, it was evident on April 29 that the treatment would not adequately protect the crop until harvest, so the field was sprayed to avoid loss to the grower.

Insecticides Effective Against Alfalfa Weevil, Pea Aphid, and Meadow Spittlebug.—In much of the Eastern area infested with the alfalfa weevil, the meadow spittlebug and the pea aphid are also important pests, and hundreds of acres are treated to control them. The necessity of mutiple-spray applications increases production costs and the residue hazard. In 1953 it was observed that the use of some of the chlorinated hydrocarbon insecticides for weevil control was followed by an increase in pea aphid population. This was probably due to the effect on the predators or parasites of the aphid. From 1954 to 1957 tests were made to determine the possibility of controlling all three insects with a single treatment. Data were taken on all the insecticides listed in table 1, but only those for the materials showing promise for control of all three pests are summarized in table 7. At the dosages used endrin, Phosdrin®, (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) and Thiodan were somewhat better than the others. However, equally good or better control was obtained with mixtures of insecticides.

Conclusion.—These investigations show that satisfactory control of alfalfa weevil larvae in the East requires an insecticide that is effective for 3 to 4 weeks. Aldrin, dieldrin, endrin, heptachlor, and Thiodan are the most promising, but none of them provide complete protection for this length of time. Recommendations for their use are dependent on residue tolerances on alfalfa forage. Careful timing is necessary to control weevil larvae with one application. Good control can be obtained by treating with heptachlor at 4 ounces per acre in late April, when the plants are 10 to 15 inches tall and most of the tips are infested. If granulated materials are used, 1 to 1½ pounds of insecticide per acre will be neces-

Table 7.—Effect of different insecticides on the alfalfa weevil, pea aphid, and meadow spittlebug, 1954–57.

Insecticide	Ounces per Acre	Year Tested	Per Cent Reduction		
			Alfalfa Weevil	Pea Aphid	Spittle-bug
Endrin	8	1954	91	97	100
	.5	1957	98	88	100
Perthane	24	1955	91	80	88
Malathion	16	1955	80	94	78
Phosdrin	4	1957	100	97	56
	8	1957	95	92	72
Thiodan	8	1957	98	67	100
	16	1957	97	33	100
Heptachlor+malathion	4+10	1955	100	81	100
Heptachlor+demeton	4+ 4	1956	100	98	100
Heptachlor+Diazinon	4+16	1956	100	99	93
Dieldrin+malathion	4+10	1956	100	98	96
Aldrin+malathion	6+10	1956	99	95	100
Heptachlor+dicapthon	4+ 8	1957	100	90	88

sary, which will considerably increase the cost. A few insecticides showed some control of the pea aphid and meadow spittlebug in addition to the alfalfa weevil, but to obtain control of all three pests a mixture of insecticides is more satisfactory.

References Cited

App, B. A. 1954. Insecticide tests to control the alfalfa weevil and meadow spittlebug in Maryland. Jour. Econ. Ent. **47**(2): 313–6.

Blackburn, Norris D. 1957. Effects of some insecticidal materials on larval populations of the alfalfa weevil in southern Pennsylvania. Jour. Econ. Ent. **50**(5): 559–62.

Manglitz, G. R., and B. A. App. 1957. Biology and seasonal development of the alfalfa weevil in Maryland. Jour. Econ. Ent. **50**(6): 810–3.

Muka, Arthus A. 1957. Alfalfa weevil control with granulated insecticides in Virginia. Jour. Econ. Ent. **50**(2): 216–8.

Walstrom, R. J., and J. A. Lofgren. 1957. Granulated insecticides for control of the alfalfa weevil. Jour. Econ. Ent. **50**(5): 574–5.

The Utility of Field Tests for Evaluating Insecticides Against the Garden Symphylid[1]

Angus J. Howitt,[2] J. S. Waterhouse,[3] and R. M. Bullock[4,5]

Abstract

From 1955 to 1957 a total of 54 acres were established in field plots to evaluate compounds for the control of *Scutigerella immaculata* Newport. Productive results were obtained in approximately 10% of the plots. Interpretation of results was complicated in the remainder of the plots by the irregular distribution pattern, non-feeding phases, and preferential feeding habits of the pest. Although it failed to give control in some trials, parathion used as a soil treatment at the rate of 5 pounds of active ingredient per acre consistently gave better results than any other insecticides tested.

The garden symphlid (fig. 1) *Scutigerella immaculata* (Newport) is a major pest of vegetables, small fruit, ornamentals, nursery, and certain specialty crops such as mint and hops in Washington and Oregon. Previously, symphylids were considered a pest in the Pacific Northwest only west of the Cascade Range where a cool, moist, oceanic type of climate prevails. In the past 4 years, however, symphylids have caused considerable damage east of the Cascade Range, where warm, dry, continental climatic conditions exist. Symphylid damage east of the Cascades was reported in 1946 but it was not considered a serious pest in these areas then.

The garden symphlid is a general feeder attacking most plants. It chews through the seed coats (fig. 2), riddles the seed, and destroys the embryo. This type of injury causes serious reduction in stand. The pests feed on the root system eating root hairs and rootlets (fig. 3). The larger roots may be pitted from feeding. In many instances, all that remains is a skeletal root system devoid of fine roots. Affected plants not killed outright are stunted, mature late, and give low yields of inferior quality. Root crops such as radish, beets, carrots, and turnips may be rendered unmarketable by the small holes chewed in their skin and tissues. Parts of plants above the soil may be riddled with holes if they are in contract with the ground. Crops such as asparagus and celery are made unsalable by holes in the stalks and the rusty corky outgrowths which often develop at sites of injury. Ripe strawberries in contact with the soil are damaged when symphylids tunnel through the fruit.

Howitt & Bullock (1955) reported that in field tests using soil treatments the chlorinated hydrocarbons were generally ineffective, while promising results were obtained with parathion. The present study was undertaken

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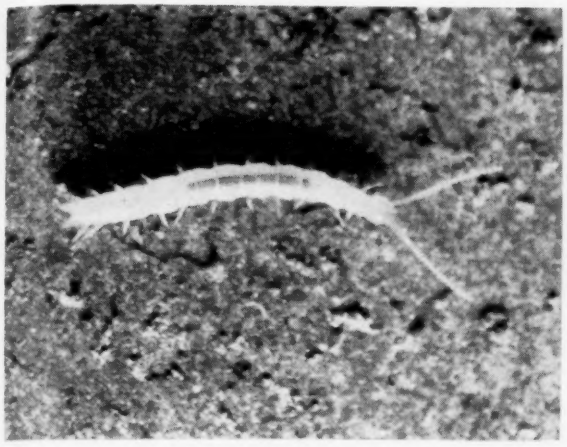

FIG. 1.—An adult symphylid.

to make additional field evaluations with parathion and other toxicants in the control of the garden symphlid. The chemical definitions of the compounds referred to in this and in the two following papers, except for those that appear in "Common Names of Insecticides" (see JOUR. ECON ENT. **52**(2): 361–2, 1959) are as follows:

Am. Cyanamid 12008 = *O,O*-diethyl *S*-(isopropylthio)methyl phosphorodithioate

Am. Cyanamid E. N. 18133 = *O,O*-diethyl *O*-2-pyrazinyl phosphorothioate

FIG. 2.—Symphylid damage to sprouting bean seed.

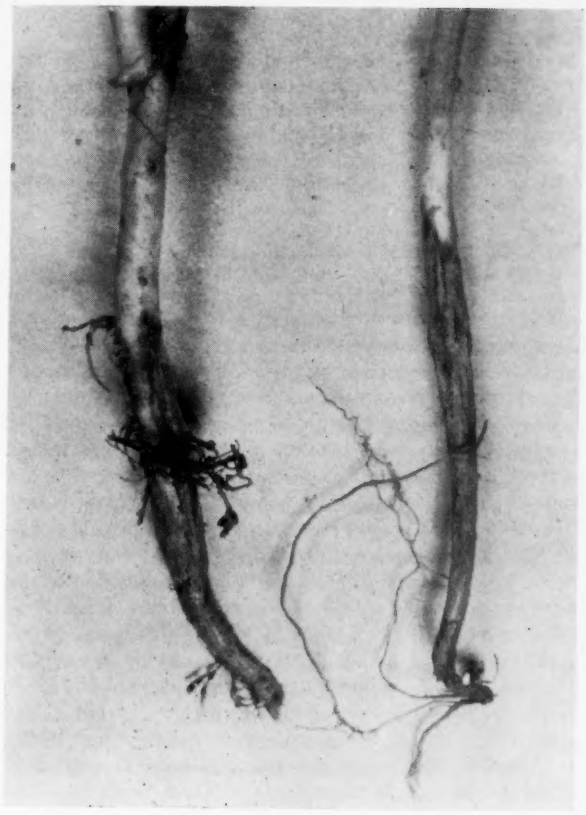

FIG. 3.—Bean plants, devoid of root hairs and rootlets caused by symphylid feeding.

Aramite® = 2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite

Chlorobenzilate® = ethyl 4,4′-dichlorobenzilate

Chlorthion® = *O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate

Co-Ral® = *O*-(3-chloro-4-methylumbilliferone)*O,O*-diethyl phosphorothioate

DBCP = 1,2-dibromo-3-chloropropane

D-D mixture® = mixture of 1,2-dichloropropane and 1,3-dichloropropene

Delnav® = 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate)

Diazinon® = *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate

Dibrom® = *O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate

Dipterex® = *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate

Di-syston® = *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate

Genite® = 2,4-dichlorophenyl benzenesulfonate

Guthion® = *O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl)phosphorodithioate

Kelthane® = 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol

Kepone® = 1,2,3,5,6,7,8,9,10,10-Decachlorotetracyclo [5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$] decan-4-one

Mylone® = 3,5-dimethyltetrahydro-1,3,5,2 H-thiadiazine-2-thione

Perthane® = 1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane

PVP Iodine-10 (Polvinylpyrrolidone 90%, iodine 10%)

Sevin® = 1-naphthyl-*N*-methyl carbamate

Telone® = dichloropropenes mixture

Tetram® = *O,O*-diethyl *S*-(2-diethylamino)-ethyl phosphorothioate [hydrogen oxalate salt]
Thimet® = *O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate
Thiodan® = 6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
Trithion® = *S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate
UCC 8305 = P-chloro-2,4-dioxa-5-methyl-P-thione-3-phosphabicyclo (4.4.0) decane
Vapam® = Sodium methyldithiocarbamate
Vatsol OT 70% liquid = Sodium dioctyl sulfo-succinate
V-C 13 = *O*-2,4 dichlorophenyl *O,O*-diethyl phosphorothioate

METHODS.—Emulsion concentrates and wettable powders were applied with a 20-foot boom sprayer. Thorough mixing with soil was accomplished by applying materials in a split or single application followed by "rototilling" or multiple discing. With split applications one-half of the dosage was applied, then the plots were disked, cross-disked, and plowed. The remaining dosage was applied immediately afterward and the plots were again disked, Morrison (1954) reported that, lacking Rototillers, this method of tillage gave the best distribution of insecticides in the soil. Granular DBCP Test 10, was applied in bands, 8 inches apart at a depth of 8 inches using a John Deere grassland drill. Plots sprayed with PVP-Iodine-10 received no tillage. Immediately after spraying, these plots were irrigated for 8 hours so that the water-soluble PVP-Iodine-10 would be carried into the soil.

Morrison (1954), Kiigemagi *et al.* (1958), and Bollen *et al.* (1958) reported on the inactivation or decline of insecticides in soil. In cognizance of these findings, equal weights of aldrin and urea were mixed together to stabilize the aldrin.

Hornstein & Sullivan (1955) reported that chlorinated terphenyls (available commercially as Arochlor 5460) extended the toxic life of parathion, malathion, Dipterex®, Diazinon®, and DDVP in laboratory tests. Chlorinated terphenyls were added to these toxicants in Tests 3, 5, and 6 to test them under field conditions. The following proportions, based on a weight-volume basis, were employed: Insecticide 10%, chlorinated terphenyls 20%, Triton X-100 10%, and xylene 60%. The resinlike chlorinated terphenyls were dissolved in the xylene, then the emulsifying agent and insecticide were added.

Evaluation of compounds for symphylid control under field conditions is extremely difficult. Two criteria were employed: presence of symphlids and crop response. Estimating symphylid populations is difficult, owing to their irregular distribution pattern. Edwards (1957) reported that symphylid distribution is usually uneven, with large populations concentrated in different areas. Savos (1958) reported that the tendency of symphlids to occur in aggregates complicated sampling designed to estimate populations. Average counts, then, have doubtful value since zero readings can be recorded for many sampling sites including those in untreated plots. Since sampling for symphylids is a tedious process, it was not possible to obtain sufficient samples for statistical analysis. Therefore, emphasis was placed on the presence rather than the absence of symphylids in treated plots. All treatments were examined repeatedly during the season. In sampling, approximately one-quarter of a cubic foot of soil, 12 inches deep, selected at random was removed and the number of symphylids recorded. The presence of symphylids in treatments could be emphasized by reading only the three highest counts of symphylids per sample for each month during the course of the experiment. The figures in the tables listed as average number of symphylids were derived by averaging the three highest counts for each month. Savos (1958) reported that most symphylids were found 6 to 12 inches in the soil, therefore sampling to 12 inches minimized the variations in numbers due to vertical distribution.

A number of problems are presented in the matter of crop response. Although symphylids are considered omnivorous feeders, they will attack some crops more readily than others. Field evaluations are further complicated by the existence of feeding cycles. Michelbacher (1938), Edwards (1957), and Savos (1958) have reported feeding was usually more pronounced during the first part of the feeding cycle.

Edwards (1957) reported that at any one time, no more than two-thirds of the symphylids present in the soil are likely to be in a feeding phase. He stated that a symphylid was capable of eating 15 times its own weight within a 24-hour period. Since the daily amount of food consumed by a single individual is relatively large, and since nonfeeding symphylids leave the plant for other soil strata, it is obvious that plant response and numbers of symphylids present per sample will not show a constant relationship. Since the presence of symphylids is not necessarily correlated with plant injury, data are presented in the tables on both plant growth and symphylid numbers.

RESULTS.—Results of 13 tests are presented in table 1. Tests 1 and 2 were designed to determine if conditions under which the toxicants were applied were a limiting factor in their effectiveness. Parathion, which had given promising results in earlier trials reported by Howitt & Bullock (1955), was tested with other toxicants under favorable and unfavorable field conditions. In Test 1, the insecticides were applied early in the year when the soil was wet and cold and had a tendency to lump so that perhaps the soil and insecticides were poorly mixed. In addition, the symphylids were in the lower soil levels at the time of treatment. Test 2 was applied under ideal conditions in July when the soil was friable and the symphylids in the upper soil level. It was anticipated that all toxicants, including parathion, would be ineffective when applied under the conditions of test 1. It was expected that superior performances might be expected for test 2.

Table 1.—Maximum number of symphylids per sample and the ratings for plant growth in plots treated for the control of the garden symphylid, 1955–1957, inclusive.

CHEMICAL	POUNDS OF ACTIVE INGREDIENT PER ACRE	NO. OF REPLICATES	PLOT SIZE (ACRES)	MAXIMUM NO. OF SYMPHYLIDS PER SAMPLE	PLANT GROWTH
Test 1—corn, squash, treated May 12, 1955. Parkrose, Oreg.					
Parathion	5	4	2.2	3.1	normal
Malathion	20	4	3.0	19.1	poor
Untreated	—	3	1.5	14.0	poor
Test 2—tomatoes, treated July 1955. Parkrose, Oreg.					
Aldrin+urea	7.5	2	0.6	9.5	poor
Diazinon	15	2	.6	3.5	poor
Chlorthion®	15	2	.6	22.0	poor
Parathion	10	2	.6	3.0	poor
Aldrin	7.5	2	.6	18.5	poor
Malathion	20	2	.6	51.0	poor
Untreated	—	4	1.2	12.5	poor

Table 1.—(*Continued next page*)

Table 1.—(*Continued*)

CHEMICAL	POUNDS OF ACTIVE INGREDIENT PER ACRE	NO. OF REPLICATES	PLOT SIZE (ACRES)	MAXIMUM NO. OF SYMPHYLIDS PER SAMPLE	PLANT GROWTH
Test 3—pepper, cabbage, carrots, treated June 1955. Parkrose, Oreg.					
Parathion	5	3	0.75	0	normal
Parathion +chlorinated terphenyl	5	3	.75	2.7	normal
Malathion	5	3	.75	0.5	normal
Malathion +chlorinated terphenyl	10	3	.75	4.6	normal
Dipterex	10	3	.75	4.3	normal
Untreated	—	3	.75	2.3	normal
Test 4—cabbage, treated June 1955. Parkrose, Oreg.					
Aldrin+urea	10	3	1.7	5.0	poor
Malathion	20	3	1.7	34.5	poor
Parathion	5	3	1.7	5.5	fair
Untreated	—	4	4.0	8.5	poor
Test 5—corn, treated May 26, 1955. Vancouver, Wash.					
DDVP+chlorinated terphenyl	5	6	0.30		normal
Aldrin+urea	10	6	.30		normal
Delnav®	10	6	.30		normal
V-C 13	75	6	.30		normal
DBCP	970	6	.30		normal
DDVP	5	6	.30		normal
Chlorthion	10	6	.30		normal
Thimet	10	6	.30		normal
Am. Cyanamid 12008	10	6	.30		normal
EPN	10	6	.30		normal
Diazinon	10	6	.30		normal
Malathion	10	6	.30		normal
Parathion +chlorinated terphenyl	5	6	.30		normal
Parathion	10	6	.30		normal
Test 6—corn, cucumbers, treated June 9, 1955. Longview, Wash.					
Parathion	5	2	0.25		normal
Parathion +chlorinated terphenyl	5	2	.25		normal
Malathion	10	2	.25		normal
Malathion +chlorinated terphenyl	10	2	.25		normal
Untreated	—	4	.50		normal
Test 7—sugar beets, treated May 2, 1956. Spokane, Wash.					
Parathion	5	1	0.90	28	fair
Guthion®	4	1	.90	29	fair
Untreated	—	1	.40	50	fair
Test 8—cucumbers, treated May 14, 1956. Vancouver, Wash.					
Delnav	5	3	0.50	3.5	normal
Trithion®	5	3	.50	0.6	normal
Parathion	5	3	.50	0.6	normal
Untreated	—	3	.50	3.0	normal
Test 9—mint, beans, carrots, and cabbage, treated May 10, 1956. Fargher Lake, Wash.					
Parathion	5	1	0.20	4.8	normal
Trithion	5	1	.20	1.3	normal
Delnav	5	1	.20	6.9	normal
Untreated	—	1	.15	5.0	normal
Test 10—cabbage, treated May 11, 1956. Vancouver, Wash.					
Delnav	5	1	0.10	2.6	normal
DPCP—granules	140	1	.10	1.0	normal
Parathion	5	1	.10	2.3	normal
Trithion	5	1	.10	1.3	normal
PVP-Iodine-10	15	1	.10	2.0	normal
Untreated	—	4	.20	3.8	normal
Test 11—asparagus, treated July 1, 1956. Toppenish, Wash.					
Parathion	5	2	2		normal
Trithion	5	2	2		normal
PVP-Iodine-10	15	2	2		normal
Ethylene dibromide	8	2	1		normal
DBCP	16	2	1		normal
Guthion	5	2	2		normal
Untreated	—	2	2		normal
Test 12—mint, treated April 19, 1957. Longview, Wash.					
Parathion EC	5	1	0.50	6.4	fair
Parathion EC+Thimet	5+1 H.D.[a]	1	.50	3.4	fair
Untreated	H.D.	1	.50	19.2	poor
Parathion EC+Thimet	5+1 L.D.	1	.50	3.4	fair
Untreated	L.D.	1	.50	24.6	poor
Parathion EC	5 L.D.	1	.50	8.4	fair
Test 13—corn, treated July 9, 1957. Vancouver, Wash.					
Parathion EC	5	2		0.10	poor
UCC 8305 WP	5	2		0.10	poor
Untreated	—	2		0.10	poor

[a] H. D. and L. D. refer to heavy and light disking, respectively.

Surprisingly, the reverse was true. All compounds, including parathion, failed to give any control when applied under the conditions of test 2. Conversely, parathion gave outstanding control under the conditions of test 1. It is possible that the poor plant response in test 2 could be attributed, in part, to climatic conditions since the season was wet, cold, and generally unfavorable for tomatoes. The crop response in test 1 (fig. 4) presents a good example of the singular difficulties involved in evaluating compounds against symphylids by means of plant response. The northern and southern halves of each plot had been planted to squash and corn, respectively. An excellent crop of squash, without evidence of symphylid damage, was harvested in the parathion-treated plots whereas squash in the malathion-treated and untreated plots was destroyed almost to the last plant. Growth of corn in plots treated with malathion or parathion, and in untreated plots was normal even though there were high populations of symphylids in the malathion-treated and untreated plots. In this instance, squash was the preferred crop. However, their preference for a crop may change within a season, or from season to season. Corn is generally preferred, and very susceptible to symphylid attack.

Test 3 was established to determine if addition of chlorinated terphenyls would extend the residual effectiveness of organic phosphates. Symphylid populations failed to develop in sufficient numbers in these plots to evaluate the compounds.

In Test 4, a large "target area" approximately 250 feet in diameter developed near the center of the field (fig. 5). A single replication of plots treated with aldrin plus urea, aldrin, or parathion, and one untreated plot was within this endemic area. Cabbage plants in the plots treated with malathion, or aldrin plus urea, and the untreated plot, were either killed outright or severely stunted. Cabbage plants in the parathion-treated plot had some injury, although much less than in other infested plots. As the season progressed, these differences became more apparent. Later in the season these cabbage plants had noticeably more vigor and growth than plants in the adjoining plots. Unfortunately, an early season frost killed all plants and data on yields were not obtained.

Test 5 inadvertently demonstrated the futility of using small plots to evaluate toxicants in the field. This experiment was designed to screen a large number of compounds for symphylid control. A total of 300 separate plots, each measuring $10' \times 25'$, were treated. Considerable time, labor, and special equipment were involved. Although symphylid damage appeared in some plots, the results were inconsistent and the findings had to be considered inconclusive.

Treatments in test 6 were applied after the grower had lost an early planting of beans. Two adjoining fields were treated, then planted to corn and cucumbers. Favorable yields were obtained from both fields, so the grower was convinced the treatments were effective. However, the lack of symphylids and good growth of crops in the untreated plots indicated that these tests were inconclusive.

In Test 7 the soil was a gravelly loam-stony phase so that it was difficult to obtain a proper soil-insecticide mix. Symphylids had caused severe losses in the previous year and at the time of treatment numerous symphylids were observed. Although high populations were present

FIG. 4.—Field results showing squash in the malathion-treated plot to the right of the stake in the foreground severely damaged by symphylids, while the squash in the parathion-treated plot to the left of the stake is undamaged. Corn in the background shows no indication of injury in both the malathion- and parathion-treated plots.

throughout the growing season, there was little evidence of symphylid damage. There was no explanation for these phenomena, although in view of the large number of symphylids present, the treatments could not be considered successful.

In Test 8 the crops had been severely damaged in previous years. At the time of treatment, numerous symphylids were present near the surface of the soil. There was no evidence of symphylid injury and very few symphylids were observed in any of the plots, including the checks. The field was not irrigated so the soil remained quite dry throughout the summer. This may have accounted for the lack of symphylid damage. In some instances, crops have been grown successfully on symphylid-infested soil when the soil has contained minimum amounts of moisture necessary for plant development, but not enough for symphylid activity.

In Test 9 the plots were established in muck soil which had been planted to mint. Growth of the mint was sparse and stunted from symphylid feeding. At the time of treatment symphylids were numerous in the upper soil strata. One-half of the field was planted to cabbage, carrots, and beans, while the remainder was left in mint. Row crops in all treated and untreated plots grew normally with no evidence of symphylid feeding even though symphylids were present in sufficient numbers to cause damage.

In Test 10 the growth of cabbage in all plots was normal. There was no indication of symphylid feeding on the roots in the treated and untreated plots. Previous crops in this field, including asparagus and raspberries, had been severely injured by symphylids.

In Test 11 although treatments were applied in the summer of 1956, they could not be evaluated until the spring of 1957. When asparagus spears were examined in the fall of 1956, it became evident that it was impossible to distinguish whether feeding punctures occurred before or after treatment. Since evaluation of treatments was based on the number of punctures in the spears, it was decided that evaluation should be made on new growth the following spring when symphylid damage would have taken place after treatment. There was no significant difference between treatments.

In Test 12 the toxicants were worked into the mint field by deep and shallow disking to determine the effects of tillage on an established field of mint. Though deep thorough mixing of insecticides into the soil is desirable for symphylid control, it was not known how these methods would affect mint. Some untreated plots of mint were left untilled, and some were cultivated by deep diskings. Sampling after treatment showed a marked reduction in symphylids in all treated plots compared with untreated areas. The mixture of parathion and Thimet® appeared to reduce the number of symphylids more than parathion used alone. In 1958, mint growth in all treated plots appeared to be more vigorous than mint in the untreated areas. There was no difference in growth between the shallow- and deep-cultivated plots. Neither method of tillage appeared to have an adverse effect on the growth of mint.

In Test 13, 2 weeks after treatment, many symphylids were observed in all plots. Corn in the treated and untreated areas was severely damaged. Soil samples were

FIG. 5.—Field of cabbage showing "target area" of stunted growth to the left in the aldrin-treated and untreated plots. Parathion treatment is shown in the right half of the picture.

taken from the plots treated with UCC 8305 and parathion 3, 4, and 5 weeks after treatment and bioassayed with symphylids. In all cases 100% mortality was obtained. Apparently, factors other than resistance of symphylids to organic phosphates were responsible for the lack of control.

From 1955 to 1957 a total of 54 acres were established in symphylid plots to evaluate soil insecticides for symphylid control. In 42.6% of these plots, inconclusive results were obtained because symphylid populations did not develop, even though plots were established in fields known to be infested. Of the 57.4% of the plots where symphylid populations developed, 46.3% were not productive since factors such as the irregular distribution pattern, nonfeeding phases, and preferential feeding habits of the pest complicated the interpretation of results. Productive findings then were obtained in only 11.1% of the plots treated. It is evident that comparative evaluation of compounds for symphylid control under field conditions is extremely difficult under these conditions. When a compound is effective, its effectiveness will be demonstrated by repeated successful applications. For example, parathion has been used with considerable success since 1955. In 1956, 500 acres out of a total of approximately 3,000 acres teated with parathion were inspected for symphylid damage. Symphylid damage occurred on approximately 2% of the acreage treated. However, an increasing number of failures with parathion have been reported in the past 2 years.

Unfortunately, when a compound is ineffective, it may require several years of field testing to determine that it has little value for symphylid control. As mentioned earlier, an ineffective toxicant could give apparent control 88.9% of the time owing to the factors listed previously. A method is needed whereby candidate materials may be screened under controlled conditions to determine if they should be evaluated in the field.

A study of the ecology of *Scutigerella immaculata* to determine the reasons for the marked changes in population levels and the factors that contribute to its erratic behaviour would be of great value in control programs. To date, there is a paucity of literature concerned with ecological studies of this pest.

REFERENCES CITED

Bollen, W. B., J. E. Roberts and H. E. Morrison. 1958. Soil properties and factors influencing aldrin-dieldrin recovery and transformation. Jour. Econ. Ent. **51**(2): 214–9.

Edwards, C. A. 1957. Bionomics of symphylid. Unpublished Ph.D. thesis. University of Wisconsin, Madison, Wisconsin.

Hornstein, Irvin, Wm. N. Sullivan, and Ching-hsi Tsao. 1955. Residual effectiveness of mixtures of organic phosphorous insecticides with chlorinated terpenes. Jour. Econ. Ent. **48**(4) 482–3.

Howitt, Angus J., and R. M. Bullock. 1955. Control of the garden centipede. Jour. Econ. Ent. **48**(3): 246–50.

Kiigemagi, Ulo, H. E. Morrison, J. E. Roberts and W. B.

Bollen. 1958. Biological and chemical studies on the decline of soil insecticides. Jour. Econ. Ent. 51(2): 198–204.

Michelbacher, A. E. 1938. The biology of the garden centipede, *Scutigerella immaculata*. Hilgardia 11(3): 55–148.

Morrison, H. E. 1954. A progress report on symphylid control research. Ann. Rept. Oregon State Hort. Soc. 46: 125–9.

Savos, Milton George. 1958. The bionomics of the garden symphylid. Unpublished Ph.D. thesis. Oregon State College, Corvallis, Oregon.

Laboratory and Greenhouse Tests for Evaluating Compounds in the Control of the Garden Symphylid, *Scutigerella immaculata* (Newport)[1]

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ABSTRACT

Basic toxicities of compounds against *Scutigerella immaculata* Newport were determined by bioassay methods. To simulate field conditions, bioassay tests were conducted by mixing insecticides with required amounts of soil to give mixtures that contained 2.5 p.p.m. of the insecticides. With the exception of lindane, the chlorinated hydrocarbons had little effect on this pest. Of the 46 compounds tested, only parathion, UCC 8305 (P-chloro-2,4-dioxa-5-methyl-P-thiono-3-phosphabicyclo (4.4.0) decane), V-C 13 (*O*-2,4-dichlorophenyl *O,O*-diethyl phosphorothioate), Am. Cyanamid E.N. 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate) and lindane were initially toxic and possessed some degree of residual effectiveness. Am. Cyanamid E.N. 18133 gave outstanding results and appeared to be the most promising of the insecticides tested.

Edwards (1957), Savos (1958) and Morrison (1954) reported the difficulties of evaluating insecticides in the control of the garden symphylid under field conditions. Howitt *et al.* (1959) reported that in 54 acres of experimental plots, conclusive results were obtained in only 11.1% of them. Since it was extremely difficult to evaluate compounds in the field, controlled laboratory experiments were conducted to determine, as fully as possible, the potential value of toxicants in the control of symphylids. In this way only promising compounds would be field tested so that the hazard of testing compounds for a number of years in the field before determining their value would be eliminated.

METHODS.—To determine basic toxicities of insecticides against the symphylid under simulated field conditions, all insecticides were mixed with required amounts of soil to give mixtures that contained 2.5 p.p.m. of the insecticides. This represented a dosage of 5 pounds actual toxicant per acre ½ foot, assuming that an acre ¼ foot of garden soil weighs approximately 1 million pounds. Melbourne clay loam soil was used in all tests. Soil samples taken fron a field classified as Melbourne clay loam, which had been sprayed with parathion at 5 pounds of actual toxicant per acre and multiple disked then analyzed immediately, yielded 2.2 p.p.m. of parathion. Soil samples taken from this field and mixed with the required amount of parathion calculated to give soil mixtures containing 2.5 p.p.m. of parathion yielded 2.6 p.p.m. upon analysis.

A Twin Shell Blender was used to insure thorough mixing of the insecticide with the soil. Only dusts or wettable powder formulations were employed, since it was believed that adsorption might hinder proper mixing if emulsion concentrates were used. To reduce errors in mixing, standard procedures were followed. To minimize errors in weighing, the initial amounts of materials were obtained by weighing 100 grams of the insecticide and 900 grams of air dried soil on a Toledo balance scale prior to mixing them in the blender. Four dilutions for dusts and five of wettable powders were used in arriving at the 2.5 p.p.m. insecticide mixture.

The Twin Blender was cleaned between mixings with a portable vacuum cleaner equipped with a 1½-horsepower motor. After the soil particles were removed, the interior of the blender was swabbed with acetone. It was found that erroneous results were obtained with materials toxic to symphylids when the blender was not cleansed between mixings. Immediately after final mixing, the insecticide-soil mixture was placed in open pint jars. Additional amounts of this same mixture were placed in open 3-gallon containers and stored outdoors. Each test was replicated three times. A replication consisted of three pint jars filled with the particular insecticide-soil mixture under test, each containing 30 symphylids or 90 per treatment. In the initial screening test, the symphylids were maintained in the jars for 30 days on lettuce leaves before mortality was recorded. During the experiments, the soil in each container was sprayed lightly with water at the surface to keep it moist. The temperature and relative humidity were maintained at 75° F. and 60%, respectively.

If results from the 30-day test were promising, further trials to determine the residual effectiveness of the compound were undertaken. In these tests, the insecticides were mixed, then stored outdoors in the manner previously described. At weekly intervals, soil samples from these containers were brought into the laboratory and bioassayed with symphylids.

Originally, symphylids were exposed to these soil-insecticide mixtures for 1 week. However, it was found

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FIG. 1.—One-quart jars on the left and right containing treated soil in the upper half and untreated soil in the lower half. A two-quart jar containing untreated soil in the middle and treated soil in the upper and lower thirds of the jar is shown in the center.

that when treated soils were stored for varying intervals before bioassay tests were conducted, the symphylids required a longer exposure period before they were affected. Accordingly, the symphylids were confined in the treated soils 2 weeks before mortality was recorded. L.T. 50's were obtained for the most promising compounds by this method. An L.T. 50 is the interval of time after the soil was treated at which 50% of the symphylid population was susceptible enough to succumb, and 50% were sufficiently resistant to survive when exposed to the treated soil.

L. D. 50's were obtained by the bioassay technique described previously, with additional dilutions to obtain the necessary range of dosages.

To relate laboratory findings with residues obtained from field applications, parathion was applied at 5 pounds of actual toxicant per acre to a field classified as Lauren clay loam. The treatment was applied with a boom sprayer using 50 gallons of water per acre. Thorough mixing of the toxicant in the soil was obtained by "rototilling" the plot immediately after treatment. The soil was sampled and analyzed for parathion 1, 7, 14, 21, 28, 62, and 79 days after treatment.

Although laboratory work indicated that some compounds had residual activity for 2 or 3 months, symphylids were often observed in the field in plots treated with these compounds within 2 weeks after treatment. For example, while the L.T. 50 of parathion, determined by laboratory methods, was 17.5 weeks, it was known that symphylids often appeared in parathion-treated plots 10 to 14 days after treatment. The following techniques were used to study this phenomenon: One- and two-quart glass containers were used (fig. 1). The lower half of the 1-quart jars (left and right jars in fig. 1) contained untreated soil while the top half contained the soil-insecticide mixture. In the 2-quart jars (center jar of fig. 1) treated soil was placed in the upper and lower one-third sections of the jar. Unteated soil were placed in the middle section. Lettuce leaves were placed at the soil surface of all jars to serve as an attractant. Using the arrangements of treated and untreated soils shown in figure 1, toxicants were tested in the following ways:

Method 1.—The symphylids, free to move, were placed on the surface of the freshly treated soils contained in the top layer of the jars.

Method 2.—The symphylids, free to move, were placed in the center of the untreated soil in the lower half of the 1-quart jars. Immediately afterward, the upper half of the container was filled with treated soil as follows: a) treated soil placed in upper one-half of jar immediately after mixing; b) treated soils placed in upper one-half of the jar after they had been stored outdoors for 7, 14, 21, 28, 60, 120, 160, and 190 days.

Method 3.—Conducted simultaneously with Method 2. Symphylids were placed in cylindrical cages measuring 4″×1″ constructed of 100-mesh brass strainer cloth, then placed in the treated soil contained in the upper one-half of the jars as follows: a) immediately after the soil was treated with insecticide; b) after the soil had been treated and stored outdoors for 7, 14, 21, 28, 60, 120, 160, and 190 days.

Method 4.—The symphylids, free to move, were placed in the center of the untreated soil contained in the middle section of the 2-quart jar at the following times: a) after freshly treated soil was placed in the lower and upper thirds of the jar; b) after soils had been treated, stored outdoors, then placed in the lower and upper thirds of jar.

Very often damage from symphylids becomes apparent on crops after planting. This is particularly true of perennials such as mint, strawberries, and raspberries, when damage from symphylids may not become evident until

FIG. 2.—Cardboard carton, circled at 1-inch intervals, containing soil; section removed by cutting across carton, showing core of soil removed from center; pint jars containing soil from the sections for bioassay.

2 to 3 years after planting. In seeking control measures, many growers made surface applications of parathion. The parathion was pumped into the irrigation lines and applied in combination with the water of overhead sprinkler irrigation. Most growers believed that parathion would penetrate deeper into wet soil than into dry soil. In practice, growers irrigated until the soil reached field capacity before introducing parathion into the irrigation system. A laboratory study was conducted to determine the possible value of this control method.

The bottoms were removed from slip-top cylindrical ice cream cartons. These 1-gallon containers, 6½ inches in diameter and 9 inches tall, were constructed of waxed cardboard. They were placed in an upright position, then individual containers were filled to a height of 6 inches with one soil type. These soil types differed in their physical and chemical analyses so that the samples varied greatly both in their sand, silt, clay and organic matter content and in their aniomic and cationic exchange capacities. In addition, there were considerable differences in the phosphate fixation capacities of the soil types.

Thimet® parathion, and BHC were chosen for testing. All three compounds had previously been tested and found to be toxic to symphylids. Parathion was the compound most commonly used by growers. Thimet was known to be an unsaturated compound extremely toxic to symphylids. BHC was chosen as an example of a completely saturated compound known to be toxic to symphylids. To simulate conditions of wetting the soil prior to surface applications of the toxicant, 3 inches of water was applied to the surface of the soil in the containers. After the gravitational water had drained off the soil column, another 3 inches of water containing the required amount of an emulsion concentrate of the insecticide to be tested was added. Parathion was used at the rate of 5 pounds of actual toxicant per acre. Thimet and BHC were tested at the rate of 10 pounds of actual toxicant per acre. In other tests, 3 inches of water containing the same amounts of toxicant was added to air-dried columns of similar soil types. All moistened soil columns were left untouched for 48 hours to allow excess water to drain. After this interval the soil columns were sectioned by cutting across the cylindrical containers at the points 1 inch apart circled with rings. The soil was taken from the centers of these sections, put into glass jars and bioassayed with symphylids (fig. 2).

For testing the value of surface applications under field conditions, symphylid-infested ground beds in two large adjoining greenhouses were treated. There was a total of 10 ground beds, each of which measured 150 by 4 feet. Alleyways, 4 feet wide, running the length of the greenhouses, separated the greenhouse beds. Each ground bed was divided to make two plots, each of which measured 75 by 4 feet. At the time of treatment, tomato plants, which were at the blossom stage, were being grown in the beds. There were four replications of each treatment. A replication consisted of a 75 by 4 foot plot. Chlorthion,® malathion, parathion and Dipterex® were tested alone and in combination with Vatsol OT 70%

Table 1.—The per cent mortality of garden symphylids in soil treated with compounds at 2.5 p.p.m. and stored for different intervals before testing. Puyallup, Washington, 1957.

Insecticide	No. of Days from Soil Treatment to Test											
	0	7	14	21	28	35	42	84	91	105	140	196
Parathion	100	100	100	100	100	100	100	100	98.8	96.6	30	—
UCC 8305	100	100	100	100	100	100	100	100	46.6	5.5	—	—
V-C 13[a] (110 p.p.m.)	100	100	100	100	100	100	100	100	—	—	—	—
Am. Cyanamid E.N. 18133	100	100	100	100	100	100	100	100	100	100	100	100
Lindane	100	100	100	97.8	94.4	94.0	92.2	83.0	—	75.3	37.7	—
Thimet	100	100	100	64.7	8.8	—	—	—	—	—	—	—
Mylone[a] (150 p.p.m.)	100	44.0	38.6	33.3	32.0	—	—	—	—	—	—	—
EPN	100	17.7	15.5	—	—	—	—	—	—	—	—	—
Delnav	100	16.6	6.6	—	—	—	—	—	—	—	—	—
Sevin	100	10.2	—	—	—	—	—	—	—	—	—	—
Ronnel	100	8.8	—	—	—	—	—	—	—	—	—	—
Ethion	98.8	62.3	20.5	30.0	17.7	—	—	—	—	—	—	—
Chlorthion	98.8	24.4	5.2	—	—	—	—	—	—	—	—	—
Schradan	97.8	16.6	14.4	7.7	—	—	—	—	—	—	—	—
Trithion	96.6	32.2	5.5	—	—	—	—	—	—	—	—	—
V-C 13	93.3	90.3	86.6	85.1	82.2	42.1	20.0	—	—	—	—	—

[a] These compounds tested at the dosage listed (p.p.m.×2 = pounds actual toxicant per acre).

Table 2.—Toxicity of insecticides to garden symphylids. Puyallup, Washington, 1957.

INSECTICIDE	L.T. 50 (WEEKS)	L.T. 90 (WEEKS)	L.D. 50 (P.P.M.)	L.D. 90 (P.P.M.)
Am. Cyanamid E.N. 18133	—	28.0+	0.24	0.49
Parathion	17.5	14.3	0.38	0.65
Lindane	12.1	6.5	2.10	4.34
UCC 8305	12.5	11.1	0.32	0.62
Thimet	—	—	0.11	0.23
Dipterex	—	—	4.81	—
V-C 13	—	—	1.42	2.25

liquid at the rate of 10 pounds of actual material per acre. Vatsol OT 70% liquid was used at the rate of 2 ounces of 85% paste per 100 gallons of water to determine if a powerful wetting agent would aid penetration of the toxicant into the soil. All materials were applied as surface applications in water used at the rate of 6,400 gallons per acre. Records of symphylid numbers and plant response were made throughout the season.

To determine if the soil type affected the performance of chlorinated hydrocarbons, quantities of soil were obtained from a field classified as Chehalis silt loam for which control of symphylids with aldrin was claimed. Soil samples were obtained from a different field of Olympic loam, where aldrin had failed to control symphylids. Both soils were mixed with aldrin at 20 pounds of actual toxicant per acre using the method previously described. Five replications of each soil type were made. A replication consisted of a pint sealer full of the soil type into which 50 symphylids were placed. Mortality was recorded after 2 weeks.

RESULTS.—Tables 1, 2, 3, 4, 5, and 6 present the data recorded in these experiments. With the exception of lindane, the chlorinated hydrocarbons appeared to have little effect on the symphylids. There seemed to be a great variation in the effects of toxicants, both in their initial and residual effectiveness. Of the compounds tested, only parathion, UCC 8305, V-C 13, Am. Cyanamid E.N. 18133, and lindane were initially toxic and possessed some degree of residual effectiveness. Thimet appeared to be the most toxic compound, although its residual effectiveness was limited. Several compounds including EPN, Mylone,® Delnav,® Sevin,® ronnel, ethion, Chlorthion, schradan, Trithion® and V-C 13, were initially effective

Table 3.—The percentage mortality of garden symphylids placed, at different intervals, in adjacent layers of untreated soil and soil treated with toxicants at 2.5 p.p.m. (5 pounds actual toxicant per acre). Puyallup, Wash. 1958.

INSECTICIDE	METHOD NO.	PER CENT MORTALITY AFTER DAYS INDICATED								
		0	7	14	21	28	60	120	160	190
Am. Cyanamid E.N. 18133	1	100	—	—	—	—	—	—	—	—
	2	91.1	35.6	55.6	62.3	45.3	—	—	73.4	—
	3	97.8	98.7	100	100	100	100	100	100	100
	4	—	—	—	—	—	91.3	—	—	100
V-C 13 (110 p.p.m.)[a]	1	100	—	—	—	—	—	—	—	—
	2	100	100	100	100	100	100	100	—	—
	3	100	100	100	100	100	—	—	—	—
V-C 13	1	100	—	—	—	—	—	—	—	—
	2	84.0	—	—	—	—	—	—	—	—
	3	92.0	76.0	—	—	—	—	—	—	—
Parathion	1	100	—	—	—	—	—	—	—	—
	2	87.2	24.3	21.1	16.0	11.0	—	—	—	—
	3	12.1	6.2	4.1	5.0	5.2	—	—	—	—
	4	86.7	34.4	—	—	—	—	—	—	—
Lindane	1	100	—	—	—	—	—	—	—	—
	2	78.2	76.0	72.0	60.0	46.3	—	—	—	—
	3	34.4	16.3	12.3	5.1	6.2	—	—	—	—
	4	73.2	30.0	—	—	—	—	—	—	—
UCC 8305	1	100	—	—	—	—	—	—	—	—
	2	75.0	38.6	12.0	8.1	4.3	—	—	—	—
	3	32.0	8.0	5.3	6.1	4.5	—	—	—	—
Thimet	1	100	—	—	—	—	—	—	—	—
	2	89.1	—	—	—	—	—	—	—	—
	3	100	—	—	—	—	—	—	—	—
EPN	1	100	—	—	—	—	—	—	—	—
	2	3.2	4.1	—	—	—	—	—	—	—
	3	18.1	4.3	—	—	—	—	—	—	—
Sevin	1	100	—	—	—	—	—	—	—	—
	2	44.8	—	—	—	—	—	—	—	—
	3	4.9	—	—	—	—	—	—	—	—

[a] 110 p.p.m. = 220 pounds of actual ingredient.

Table 4.—The physical and chemical characteristics of soils used in experiments to determine the depth of penetration of toxicants applied to the soil surface. Puyallup, Washington, 1956.

Soil Characteristics	Loam Soil Type			
	Sultan Silty Clay	Cinebar Clay	Puyallup Sandy	Melbourne Clay
Per cent sand	11	24	52	26
Per cent silt	46	49	42	42
Per cent clay	43	27	6	32
Per cent organic matter	5	19	3	5
Cationic exchange capacity	20	45	9	22
Anionic exchange capacity	12	86	8	29
Phosphate fixation capacity	36	132	19	42

but had no residual effect on symphylids. The percentage mortality for ineffective compounds, after symphylids were exposed to treated soil for 30 days, was a follows: Diazinon® 63.3%, Perthane® 54.5%, Tetram® 50.0%, methoxychlor 49.8%, Dipterex 49.3%, dicapthon 49.3%, chlordane 41.1%, PVP-Iodine-10 (25 p.p.m.) 40.0%, Mylone 37.7%, Di-syston® 36.6%, Co-ral® 35.5%, Aramite® 30.3%, Thiodan® 30.0%, cryolite 28.8%, phosphamidon 28.8%, endrin 25.5%, Dibrom® 25.3%, dieldrin 24.4%, Kepone® 20.0%, Kelthane® 17.7%, Genite® 16.6%, toxaphene 13.3%, dinitrocyclohexylphenol 12.2%, malathion 11.2%, Chlorobenzilate®10.0%, Guthion® 7.7%, PVP-I 6.6%, DDT 6.0%, aldrin 5.6%, heptachlor 5.5%, untreated 4.0% (Tables 1 and 2).

It was originally believed that initial mortality was caused, in part, by the fumigation effects of the toxicant. With the exception of Thimet, V-C 13 and Am. Cyanamid E. N. 18133, the results obtained by caging symphylids in freshly mixed soil disproved this theory since there was little mortality of symphylids confined in the newly treated soils. The findings shown in table 3 explained why

Table 5.—The percentage mortality of garden symphylids when bioassayed with soils sampled from different depths after treatment at the surface with toxicants mixed with varying amounts of water. Puyallup, Washington, 1956.

Soil Type	Per Cent Mortality at Depth Indicated (inches)			
	0–1	1–2	2–3	3–4
BHC—10 lb. actual toxicant per acre				
Sultan silty clay loam—wet[a]	100	0	0	0
Puyallup sandy loam—wet	100	3.7	0	0
Cinebar clay loam—wet	100	0	0	0
Sultan silty clay loam—dry[b]	100	88.8	0	0
Puyallup sandy loam—dry	100	93.0	0	0
Cinebar clay loam—dry	100	9.3	0	0
Thimet 10 lb. actual toxicant per acre				
Sultan silty clay loam—wet	100	96.1	0	0
Puyallup sandy loam—wet	100	89.3	85.3	0
Cinebar clay loam—wet	100	7.0	0	0
Sultan silty clay loam—dry	100	96.2	93.5	0
Puyallup sandy loam—dry	100	96.0	93.4	0
Cinebar clay loam—dry	100	89.1	0	0
Parathion 5 lb. actual toxicant per acre				
Melbourne clay loam—wet	100	1.0	0	0
Melbourne clay loam—dry	100	0	0	0

[a] Wet—Prior to adding the 3 inches of water containing the required amount of toxicant the soil column had been wetted to field capacity.

[b] Dry—The 3 inches of water containing the required amount of toxicant was added to a column of air-dried soil.

Table 6.—The percentage mortality of garden symphylids in Olympic loam and Chehalis silty loam treated with aldrin at 10 p.p.m. (20 pounds actual ingredient per acre). Puyallup, Washington, 1956.

Loam Soil Type	No. of Live Symphylids (max. 250)	Per Cent Mortality
Olympic	165	34.0
Chehalis silty	180	28.0
Chehalis silty	239	4.6
Olympic	235	6.0

laboratory results indicated residual activity for parathion of 3 or 4 months while symphylids were observed in parathion-treated fields 2 weeks after treatment. When symphylids were placed in containers of parathion-treated soil, they were always in contact with the toxicant so that mortality resulted from the cumulative effects of the pesticide.

The findings indicated that single exposures to freshly treated soils were lethal to symphylids. However, as the treated soil aged, it required longer exposures because the toxicant deteriorated. This was particularly true of the organic phosphates which have limited residual effectiveness. The effects of organic phosphates, though cumulative over short periods, are reversible over longer intervals. The symphylid traveled rapidly from treated to nontreated soils. Hence, it failed to receive a lethal exposure from the cumulative effects of the insecticide. The laboratory trials approximated the conditions and results of the fields treated with parathion.

When a field was sprayed with parathion, it was multiple-disked to insure thorough incorporation of the toxicant in the soil. Tillage destroyed the runways in the upper 6 to 8 inches of soil, so that it was approximately 2 weeks before symphylids in the lower soil levels were able to enter the treated area. By this time, the parathion had so deteriorated that single exposures were not lethal and little or no mortality resulted from the rapid migration of the symphylid from the treated to nontreated soil layers.

It was apparent then, that L.T. 50 results obtained for treated soils in which the symphylids were forcibly confined, were not valid when the symphylid could move into untreated earth. The results shown in figure 3 substantiate these findings. Parathion residues of 0.2 p.p.m. obtained 79 days after the field had been treated, were sufficient to kill symphylids. However, symphylids were observed in this field during this time in sufficient numbers to cause damage.

The methods used to obtain the data presented in table 3 appeared most efficient for determining the usefulness of compounds in controlling symphylids under field conditions. To be effective in the field, a compound must have sufficient residual toxicity to kill symphylids with single or limited exposures for several months. In these studies, only Am. Cyanamid E. N. 18133 and V-C 13 fulfilled these conditions.

The results reported in table 3 indicated that Am. Cyanamid E. N. 18133 and V-C 13 were by far the most promising compounds tested to date for the control of symphylids. These are related compounds since both are substituted diethyl esters of thiophosphoric acid. Fur-

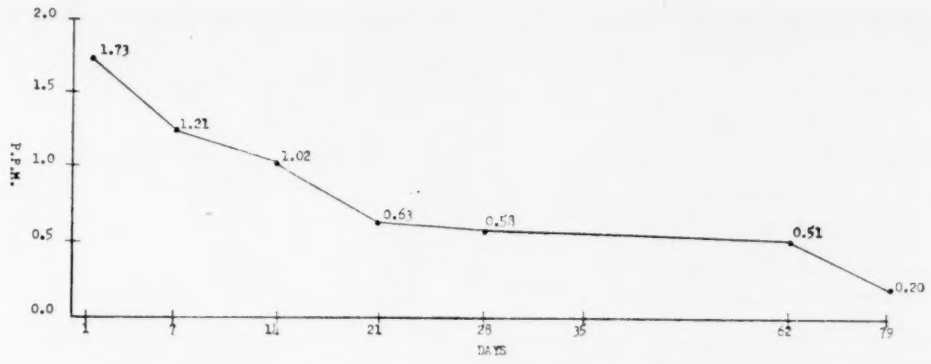

FIG. 3.—Residues, recovered at different intervals, from a field where parathion was applied at the rate of 5 pounds of active ingredient per acre as a total soil treatment.

ther tests with this group of compounds seem justified. It appeared that the fumigation effects of both toxicants persisted for a long time since symphylids caged in treated soil were still being killed after 120 and 190 days from V-C 13 and Am. Cyanamid E. N. 18133, respectively. Am. Cyanamid E. N. 18133 was more toxic than V-C 13 when tested at 2.5 p.p.m. Comparable results were obtained when Am. Cyanamid E. N. 18133 and V-C 13 were tested at 2.5 p.p.m. and 110 p.p.m., respectively. However, the L. D. 50 reported for V-C 13 indicated that this dosage was excessive so that much lower dosages might give comparable results. The apparent similarity of results obtained with parathion and Am. Cyanamid E. N. 18133, as reported in table 3 under Method 2, was caused by different factors.

The symphylids, at times, moved from the untreated to the parathion-treated soil to feed on the lettuce. The lettuce in the test with Am. Cyanamid E. N. 18133 was untouched and the surviving symphylids were usually found near the bottom of the container. It appeared that, in this case, the low mortality was due to the repellent action of this compound, and possibly to the geotropic habit of the pest. The high mortalities reported under Method 4, where the geotropic factor was eliminated and the arrangement of the soil layers increased the chances of the symphylids entering the treated soil, substantiated this opinion. The findings reported in tables 2 and 4, where mortality increased with time, further confirmed these views. It appeared that the repellent effect of the treated soil decreased with time so that after a certain interval, the symphylids entered the treated area, even though short exposures were lethal.

The results reported for V-C 13 under Method 2 indicated that soil treated with V-C 13, even at high dosages, did not repel symphylids.

It is apparent that though the penetration of compounds was deeper when they were applied to dry soils, penetration is not sufficient to exert control at any appreciable depth. The results reported in table 5 would not justify the application of water insoluble compounds as surface treatments or through the irrigation system since BHC and parathion failed to kill symphylids beyond a depth of 2 inches and Thimet was ineffective beyond 3 inches in any type of soil. A water soluble compound, toxic to symphylids, might prove useful if applied by these methods.

There was no apparent difference among treatments in growth of tomato plants or in symphylid populations in the simulated field tests conducted in the greenhouses. The tomato plants were killed or severely stunted in all plots so that no crop was harvested. Symphylid counts, made by counting the number of symphylids in approximately one-fourth cubic foot of soil, averaged 100+ per sample. As a general rule, when symphylid populations average 10 or more per sample, an economic infestation exists.

The results presented in table 6 suggest that the nonspecificity of the chlorinated hydrocarbons against the symphylid is responsible for the lack of control rather than the inactivation of the toxicant in certain soil types.

REFERENCES CITED

Edwards, C. A. 1957. Bionomics of Symphyla. Unpublished Ph.D. thesis. University of Wisconsin, Madison, Wisconsin.

Howitt, A. J., J. S. Waterhouse, and R. M. Bullock. 1959. The utility of field tests for evaluating insecticides against the garden symphylid. Jour. Econ. Ent. 52(4): 666–72.

Morrison, H. E. 1954. A progress report on symphylid control research. Ann. Rept. Oregon State Hort. Soc. 46: 125–9.

Savos, M. G. 1958. The bionomics of the garden symphylid. Unpublished Ph.D. thesis. Oregon State College, Corvallis, Oregon.

Control of *Scutigerella immaculata* (Newport) in the Pacific Northwest with Soil Fumigants[1]

ANGUS J. HOWITT[2]

ABSTRACT

Ethylene dibromide and 1,2-dibromo-3-chloropropane were ineffective in controlling the garden symphylid, *Scutigerella immaculata* (Newport), when applied to established crops. When tested under controlled conditions, methyl bromide was found to be the most effective fumigant. Of the fumigants that could be applied with a chisel-type applicator, 1,3-dichloropropene was the most effective ingredient. Chloropicrin, Vapam® (sodium methyldithiocarbamate) ethylene dibromide, 1,2-dibromo-3-chloropropane, and 1,2-dichloropropane were ineffective against symphylids when tested under controlled conditions. In controlled experiments conducted under field conditions, 10 gallons of 1,3-dichloropropene gave satisfactory control in light soils.

Attempts to control the garden symphylid *Scutigerella immaculata* (Newport) with soil fumigants have been in progress for many years. Essig (1926) and Riley (1928) reported on the use of paradichlorobenzene and carbon disulphide, respectively, in the control of this pest. Morrison & Savos (1957) reported on the use of newly introduced soil fumigants. Some of these fumigants are sufficiently nonphytotoxic to apply to certain established crops. Two criteria, plant responses and symphylid counts, are employed in evaluating compounds for the control of *S. immaculata*. Plant response is generally favorably influenced by fumigation through its effects on many factors, including fungi, bacteria, nematodes, and weeds. Therefore, the interpretation of field results with fumigants in the control of symphylids is extremely complicated. It is not surprising that conflicting results have been reported on the effectiveness of fumigants in the control of this pest in the field. The purpose of the work reported herein was four-fold:

1. To establish field trials to evaluate recently developed soil fumigants for the control of the garden symphylid.
2. To determine the effects when certain fumigants, considered to be relatively nonphytotoxic, were applied to established plantings for symphylid control.
3. To determine the relative value of different fumigants in controlling symphylids by controlled experiments.
4. To determine the most economical rates in different soil types for the fumigant most effective against symphylids.

METHODS.—Irrigation was employed when needed and in all cases the fields were cultivated before treatment so that soil moisture, tilth, and temperature were optimum for fumigation.

Evaluation of compounds in the field for the control of symphylids is difficult. Morrison (1954) reported that the peculiar feeding habits and feeding phases of symphylids limited the value of indirect measurements such as stand, yield, and vigor of a crop, while the temperature and moisture conditions limited the value of population measurements. He reported that several seasons of experimental work were wasted in establishing replicated and randomized experimental trials before it was realized that the peculiar lateral distribution of symphylids and their tendency to shift from one season to another limited the value of this type of plot design. He found that a technique which utilized nonreplicated plots from $\frac{1}{4}$ to 1 or more acres in size, in fields known to be heavily infested with symphylids, gave the best results. Replications were provided by repeating the treatments in different fields and in different localities. Howitt *et al.* (1959) reported the results of 4 years work testing chemicals for the control of symphylids under field conditions. They substantiated the findings of Morrison (1954) and demonstrated the futility of using small, randomized plots to evaluate toxicants in the field.

To date no reliable methods have been developed to estimate symphylid populations. The use of Berlese funnels and water floatation methods have not proven satisfactory. Morrison (1954) used the number of symphylids found in a garden shovel of soil as the unit of measurement. He reported that 50 or more samples were desirable in measuring the average symphylid population in a plot. He stated, however, that the accuracy of this method was reduced when the population was heavy.

Howitt *et al.* (1959) reported it was not feasible to obtain sufficient samples for statistical analysis. They reported that since zero readings could be recorded at many sampling sites, including those in untreated plots, average counts would have doubtful value unless many locations were sampled. Since it would have required the services of several individuals working steadily throughout the season to obtain the necessary number of samples, other methods of determining symphylid populations were investigated. Howitt *et al.* (1959) reported that, lacking sufficient samples, emphasis should be placed on the presence rather than the absence of symphylids in plots.

In this work, the techniques of plot design and symphylid sampling reported by Morrison (1954) and Howitt *et al.* (1959), were utilized with some modifications. There was a minimum of three replications in the controlled experiments with caged symphylids. The minimum plot size for field tests was one-third of an acre. Experiments were replicated whenever possible and in some instances paired plots were utilized. In all cases the area of invested land available determined the techniques of plot design.

In sampling, approximately one-fourth of a cubic foot of soil, 12 inches deep, selected at random was removed and the number of symphylids recorded. As many samples as possible were taken from each plot. It was de-

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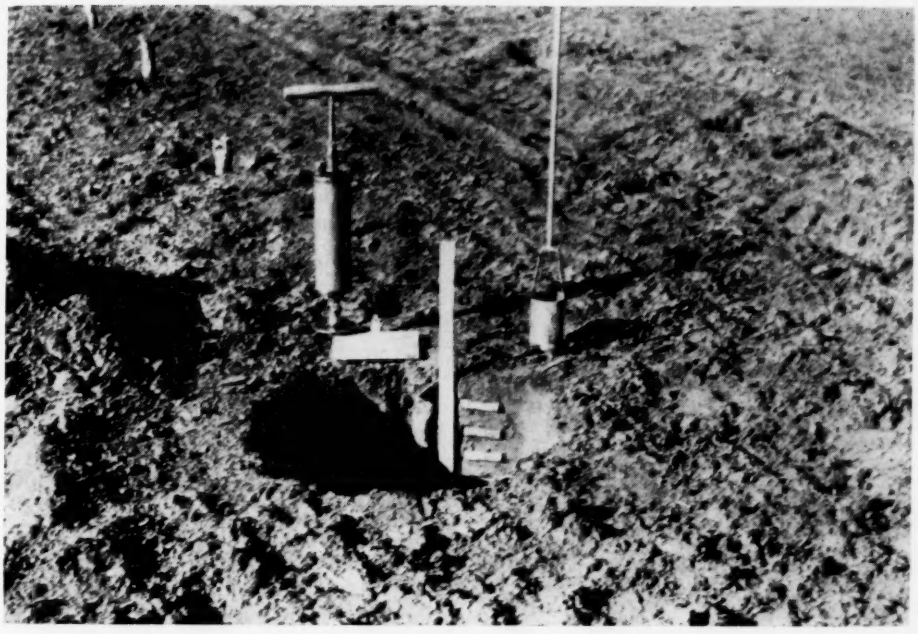

FIG. 1.—Equipment, including soil auger, MacLean Fumi-gun, and 10-inch-square board used in testing fumigants for control of the garden symphylid. The cages, containing symphylids, have been placed in the excavation at 3-inch intervals.

termined that the presence of symphylids in treatments could be emphasized by reading only the five highest counts of symphylids per plot. The figures in the tables listed as the maximum number of symphylids per sample were derived by averaging the five highest counts. Admittedly, this figure does not represent a standard statistic. On the other hand, it represents the best estimate of the potential population of a pest for which, to date, no reliable methods of sampling have been devised.

Tests 1, 2, and 3.—Some treatments were applied in September, 1955 and some in April, 1956. The parathion and Vapam® were applied by a boom sprayer. Immediately after application, the treatments were "rototilled" into the soil to a depth of 8 inches. A John Deere grassland drill was used to sow the granulated DBCP into the soil in rows 8 inches apart at a depth of 8 to 9 inches. After treatment, the soil surface was compacted with a Cultipacker. In test no. 1, both vine and bean yields were recorded.

Test 4.—The sprinkler heads were set up 40 by 40 feet apart so that all areas were thoroughly covered. The Vapam was pumped into the irrigation system from two 50-gallon drums filled with the required amount of Vapam and water. A colored dye was added to the drums to check the dispersion of the fumigant over the plot. The black raspberries were in a delayed dormant stage at the time of application.

Tests 5 and 6.—All fumigants were applied as liquids with a chisel-type applicator with the chisels spaced 14 inches apart. In applying the fumigants, the strawberry rows were straddled so that the materials were sidedressed 7 inches from the center of the row on each side. A third chisel was centered in the middle of the row 21 inches from the center of the adjoining row. A single replication consisted of seven adjacent rows of strawberries. In tests 5 and 6, 2-year-old Shasta and Marshall plants, respectively, in active stage of growth, were involved.

Tests 7 and 8.—Treatments were applied in April when the mint was just beginning to break through the ground. The soils in Tests 7 and 8 were classified as Dupont muck and Olympic silty clay loam, respectively. The growth of mint was sparse because of symphylid damage. Prior to treatments, the fields were disked 4 to 5 inches deep. In Test 7, a John Deere grassland drill was used to sow the granular materials in rows 8 inches apart at a depth of 4 inches. When the drill was set at a lower depth, it uprooted too many mint roots. In Test 8, a chisel applicator with chisels set 12″ apart was employed to place the liquid fumigants at a depth of 6 inches. The soil was computed with a Cultipacker in all cases after the application of fumigants. Symphylid counts were made throughout the growing season.

Because of the aforementioned difficulties of evaluating fumigants for symphylid control under field conditions, controlled experiments were set up. The following methods were employed to simulate field conditions as closely as possible: Three fields with widely different soil types were chosen. In one, the soil was a true peat having a high organic content. In another, the soil was a fine clay. The soil in the third field was Puyallup sandy loam. Cylindrical cages measuring 4×1 inches, constructed of 100 mesh brass strainer cloth, were filled with soil; then 25 symphylids were added to each cage. These cages were set in each field into excavations 4 inches in diameter and 24 inches deep made by a soil auger. The cages were set, with soil tamped between them, in horizontal positions at 3, 6, 9, 12, 18, 21, and 24 inches below the soil surface. A high water table prevented placement of the cages at the lower levels in the peat and clay soils. There were three replications for each dosage of fumigant tested. The fumigants were injected 8 inches deep with a MacLean Fumi-gun at the corners of a 10-inch-square board centered over the

Table 1.—Numbers of symphylids and yield or plant response in plots treated with chemicals for the control of symphylids.

Chemical	Dosage (lb. actual/A.)	Date of Treatment	No. of Replications	Plot Size (A.)	Maximum No. Symphylids/Sample	Yield[a] or Plant Growth
Test 1—bush beans. Vancouver, Wash.						
Vapam E.C.	40	Sept. 1955	2	0.5	5.0	1010—B 735—V
Parathion E.C.	5	Sept. 1955	2	0.5	2.0	847—B 757—V
Untreated	—	—	2	0.5	2.1	875—B 863—V
DBCP granules	35	Sept. 1955	2	0.5	1.5	1060—B 791—V
Parathion E.C.	10	Sept. 1955	2	0.5	0.3	1375—B 785—V
Untreated	—	—	2	0.5	3.4	1188—B 812—V
Test 2—corn. Vancouver, Wash.						
Vapam E.C.	40	Sept. 1955	2	0.5	8.7	2080
Parathion E.C.	5	Sept. 1955	2	0.5	0.5	2090
Untreated	—	—	2	0.5	5.3	1870
DBCP granules	35	Sept. 1955 April 1956	2	0.5	5.7	2310
Parathion E.C.	10	Sept. 1955 April 1956	2	0.5	1.0	2020
Untreated	—	—	2	0.5	4.0	2090
Test 3—pole beans. Vancouver, Wash.						
Parathion E.C.	10	Sept. 1955 April 1956	2	0.5	4.5	581
Parathion E.C.	5	Sept. 1955	2	0.5	4.9	580
DBCP granules	17.5	April 1956	2	0.5	28.5	642
DBCP granules	35	April 1956	2	0.5	11.4	604
Untreated	—	—	2	0.5	34.8	592
Test 4—black raspberries. Vancouver, Wash.						
Vapam E.C.	160	April 1956	1	1.0	numerous	poor
Untreated	—	—	1	1.0	numerous	poor
Test 5—Shasta strawberries. Walla Walla, Wash.						
EDB E.C.	120	July 1956	3	0.35	2.3	poor
DBCP E.C.	69.5	July 1956	3	0.35	2.9	poor
Untreated	—	—	3	0.35	4.5	poor
Test 6—Marshall strawberries. Vancouver, Wash.						
DBCP E.C.	17.5	May 1956	4	1.0	3.3	poor
DBCP E.C.	35.0	May 1956	4	1.0	1.6	poor
DBCP E.C.	70.0	May 1956	4	1.0	0.5	poor
EDB E.C.	72.0	May 1956	4	1.0	1.6	poor
Untreated	—	—	8	2.0	4.5	poor
Test 7—mint. Fargher Lake, Wash.						
DBCP granules	17.5	April 1956	2	0.5	26.5	poor
DBCP granules	35.0	April 1956	2	0.5	21.9	poor
Untreated	—	—	4	1.0	88.1	poor
Test 8—mint. Longview, Wash.						
DBCP E.C.	35.0	April 1957	2	0.5	14.2	poor
DBCP E.C.	70.0	April 1957	2	0.5	10.4	poor
EDB E.C.	60.0	April 1957	2	0.5	6.4	poor
EDB E.C.	120.0	April 1957	2	0.5	7.0	poor
Untreated	—	—	2	0.5	13.4	poor

[a] Yield given in pounds per treatment; B and V refer to the yield per treatment of beans and vines, respectively.

excavations where the cages were buried (fig. 1). A water seal was sprinkled over the soil surface in each test. Soil moisture and temperature were ideal at the time of fumigation. The cages were unearthed and the mortality recorded after 14 to 30 days (table 2).

When Telone® was determined to be most effective fumigant in the tests previously described, the following technique was employed to determine optimum field dosages: Using techniques previously described, cages containing symphylids were buried in well tilled soil classified as Puyallup sandy loam at 12, 15, 18, and 21 inches at random throughout the field. After the cages were placed in position, a chisel-type applicator with chisels spaced 12 inches apart was employed to apply Telone at a depth of 8 inches. Dosages of 5, 10, 15, and 30 gallons were applied in 12 foot strips. Each was applied to three randomized strips each measuring 200 by 12 feet. Each treated strip contained six locations at which symphylids were buried. Locations were marked by a long colored string tied to the cage at the 12-inch level and brought to the soil surface. Cages were unearthed and symphylid mortality recorded after 14 days (table 3).

Two field trials were carried out to determine the effects of Telone when applied to fields heavily infested with symphylids. Telone was applied at 30 gallons per acre in a 1-acre field of soil classified as a Pilchuck Loamy fine sand. An early season planting of corn and cabbage had been destroyed by symphylids. Symphylid counts were made before and after treatment in both the treated and untreated plots. Cages containing symphylids were buried 12, 15, 18, and 21 inches below the soil surface before treating (table 4).

A 4-acre field of Felida silty clay loam soil was treated June 5, 1958. Paired plots were employed using Telone at 20, 30 and 40 gallons per acre. Single plots treated with Telone at the 20- and 40-gallon rate were cultivated with a heavy duty spring tooth cultivator to a depth of 15 inches before treatment. The rest of the plots were prepared by normal disking. All plots were tilled and irrigated before treatment so that soil tilth, moisture, and temperature were optimum for soil fumigation. A chisel applicator with chisels spaced 12 inches apart was used to place the fumigant 8 inches deep. The plots were planted to Siedl cabbage 19 days after treatment. The crop was harvested November 12, 1958. The potential symphylid population was estimated 3 months and 11 months after treatment using the methods previously described. Weed control was evaluated several weeks after treatment, using the technique reported by Rasmussen (1956). In this method, a frame measuring 5×40 inches and divided into $2\frac{1}{2} \times 2\frac{1}{2}$ inch squares was placed at several locations in each plot. At each location the number of squares containing weeds was recorded. Percentage weed cover was determined by the formula $(Sw \times 100)/St$ where Sw = squares containing weeds, and St = total squares which was 32 for each sample (table 5).

RESULTS.—Table 1 presents the results obtained in Tests 1, 2, and 3. There was no significant difference in yield between treated and untreated plots. While parathion appeared to be more effective in reducing the numbers of symphylids than Vapam or DBCP, in some instances damaging numbers survived. Vapam and DBCP appeared to have little effect on symphylids. There was little difference in the reduction of symphylids between fall and spring, spring, or fall applications of fumigant. In Test 3, the symphylid population was extremely heavy at the time of treatment. After the pole beans had germinated, high populations were observed in all plots except in the parathion-treated areas. There appeared marked differences between treatments, with growth in the DBCP and untreated plots decidedly more stunted and retarded than in the parathion treatments. An examination of the plants in the untreated areas and in those treated with DBCP indicated severe root damage from the large numbers of symphylids. As the season progressed, differences in growth became less noticeable. The soil had been kept moist at all times during the growing season by frequent overhead sprinkling. Undoubtedly, this was responsible for the survival of the damaged plants. Later in the season fewer symphylids were noted in the plots. By harvest, time little or no difference in growth could be

Table 2.—**Mortality of symphylids[a] when caged at varying depths in different soil types and treated with different dosages of fumigants. Puyallup, Washington, 1958.**

Soil Type	Fumigant	Dosage (gal./a.)	Per Cent Control at Depth Shown (inches)							
			3	6	9	12	15	18	21	24
Peat	Telone (95%)	20	9.3	42.8	68.4	36.3	0	—	—	—
Peat	Telone (95%)	40	14.7	100	100	100	23.8	—	—	—
Fine clay	Telone (95%)	20	0	63.7	100	100	0	—	—	—
Fine clay	Telone (95%)	40	0	100	100	100	0	—	—	—
Sandy loam	Telone (95%)	5	0	81.5	100	7.4	0	0	0	—
Sandy loam	Telone (95%)	8	96.0	100	100	100	25.3	0	0	—
Sandy loam	Telone (95%)	10	100	100	100	100	100	0	0	0
Sandy loam	Telone (95%)	15	100	100	100	100	100	100	10.1	0
Sandy loam	Telone (95%)	20	100	100	100	100	100	100	30.0	0
Sandy loam	Telone (95%)	40	100	100	100	100	100	100	100	0
Peat	D-D mixture	20	0	0	31.5	0	0	—	—	—
Peat	D-D mixture	40	20.4	54.2	77.9	53.7	0	—	—	—
Fine clay	D-D mixture	20	0	0	63.8	49.1	0	—	—	—
Fine clay	D-D mixture	40	0	72.4	100	100	0	—	—	—
Sandy loam	D-D mixture	20	100	100	100	100	35.7	0	0	—
Sandy loam	D-D mixture	40	100	100	100	100	100	100	30	0
Sandy loam	1,2-D[b]	20	0	0	29.1	25.3	0	0	0	—
Sandy loam	1,2-D	40	0	35.0	74.9	37.5	0	0	0	—
Sandy loam	DMDS[c]	8	75.0	100	100	100	—	—	—	—
Sandy loam	DMDS	20	100	100	100	100	100	100	63.4	—
Sandy loam	EDB (83%)	5	100	100	100	100	100	0	0	0
Sandy loam	EDB (83%)	10	100	100	100	100	0	0	0	0
Sandy loam	EDB (83%)	20	100	100	100	100	7.1	0	0	0
Sandy loam	EDB (83%)	40	100	100	100	100	100	0	0	0
Peat	EDB (83%)	20	0	0	26.3	0	0	—	—	—
Peat	EDB (83%)	40	0	0	36.9	10.1	0	—	—	—
Fine clay	EDG (83%)	20	0	0	30.5	0	0	—	—	—
Fine clay	EDB (83%)	40	0	0	62.5	30.0	0	—	—	—
Peat	Chloropicrin[d]	20	0	0	47.4	0	0	—	—	—
Peat	Chloropicrin	40	0	37.1	96.3	27.2	0	—	—	—
Fine clay	Chloropicrin	20	0	0	44.4	0	0	—	—	—
Fine clay	Chloropicrin	40	0	20.0	100	81.8	0	—	—	—
Sandy loam	Chloropicrin	20	57.1	100	100	0	0	0	0	0
Sandy loam	Chloropicrin	40	100	100	100	100	40.0	0	0	0
Peat	DBCP	20	0	0	25.3	0	0	0	0	0
Peat	DBCP	40	0	0	89.4	0	0	—	—	—
Fine clay	DBCP	20	0	0	32.4	0	0	—	—	—
Fine clay	DBCP	40	0	0	60.6	0	0	—	—	—
Sandy loam	DBCP	20	0	0	0	0	0	0	0	0
Sandy loam	DBCP	40	0	0	33.2	0	0	0	0	0
Sandy loam	DBCP	20*	0	0	0	0	0	0	0	0
Sandy loam	DBCP	40*	0	0	40.1	0	0	0	0	0
Peat	Vapam	20	0	0	0	0	0	—	—	—
Peat	Vapam	40	0	0	33.9	0	0	—	—	—
Fine clay	Vapam	20	0	0	0	0	0	—	—	—
Fine clay	Vapam	40	0	0	0	0	0	—	—	—
Sandy loam	Vapam	20	0	0	0	0	0	0	0	0
Sandy loam	Vapam	40	0	0	28.6	0	0	0	0	0
Sandy loam	CH_3Br[e]		100	100	100	100	100	100	100	—

[a] Determined by Abbott's formula. Symphylids were not unearthed until 30 days after fumigation.
[b] 1,2-D = 100% 1:2 dichloropropane.
[c] DMDS = 99% dimethyl disulfide.
[d] Chloropicrin = 99% trichloronitromethane.
[e] CH_3Br = 98% methyl bromide applied at 2 lb. per 100 square feet.

detected between plots. While early season damage by symphylids results in permanent plant injury and reduced yields in most cases, plants can recover if sufficient soil moisture is available.

In Test 4 the Vapam appeared to have no detrimental effects on the semidormant blackberry plants, but there was no reduction in the symphylid population. Large numbers of symphylids were found before and after treatment, concentrated in the root area adjacent to the crowns of the plants.

In Tests 5 and 6 it was at first believed that the inabil-

Table 3.—**The percentage control of symphylids (Abbott's formula) when caged at different depths and treated with varying dosages of Telone. Puyallup, Washington, 1958.**

Gallons of Telone/A.	Depth of Cages (inches)			
	12	15	18	21
5	8.3	0	0	0
10	100	100	100	95.2
15	100	100	100	100
30	100	100	100	100

Table 4.—The maximum number of symphylids in treated and untreated plots before and after treatment on July 2 with Telone applied at 30 gallons per acre. Puyallup, Washington, 1958.

Date Sampled	Maximum No. of Symphylids per Sample	
	Untreated	Treated
July 1, 1958	101.3	106.4
Oct. 2, 1958	36.8	1.5
Jan. 14, 1959	79.5	3.0
Mar. 13, 1959	93.7	3.5

Table 5.—The maximum number of symphylids per sample (at different intervals after treatment) weed coverage, and the yield of cabbage in plots treated with different dosages of Telone. Puyallup, Washington, 1958.

Method	Dosage (gal./a.)	Maximum No. of Symphylids/Sample After		Per Cent Weed Coverage	Marketable Cabbage (tons/a.)	Total Weight (Tons/a.)
		3 Months	11 Months			
Deep cultivated	40	1.7	7.3	4.1	13.12	18.67
Untreated	—	10.7	8.0	35.3	5.62	11.64
Normal disking	20	1.2	21.0	8.8	11.40	16.95
Untreated	—	12.0	19.9	53.1	5.26	11.17
Normal disking	40	2.7	9.3	6.7	8.71	17.45
Untreated	—	4.0	5.7	57.2	4.92	12.18
Deep cultivated	20	1.2	12.0	6.7	9.92	18.51
Untreated	—	1.2	0	57.2	5.26	8.62
Normal disking	30	0	2.0	9.3	6.85	12.90

ity of the fumigants to penetrate to the root systems, where the symphylids were concentrated, was responsible for the lack of control obtained. However, EDB (ethylene dibromide) and DBCP were also not effective in Tests 7 and 8 when applied as broadcast treatments. These compounds are probably not specific in their action against symphylids.

The results given in table 2 indicate that there is a wide range in the effectiveness of fumigants against symphylids. When tested under a polyethylene tarpaulin under the conditions described, methyl bromide was the most effective fumigant. Of those that could be applied with conventional fumigation equipment, Telone produced the best results. Dimethyl disulphide, while not quite as effective as Telone, gave excellent control. Further work with this compound is justified because preliminary work indicated that it could be used on a number of established crops without harmful effects.

The two ingredients of D-D mixture® were tested separately. The 1,2-dichloropropane component was ineffective against symphylids at all dosages tested. Apparently, the effectiveness of D-D mixture in controlling symphylids was directly correlated with the amount of 1,3-dichloropropene used.

EDB, chloropicrin, DBCP, and Vapam were ineffective at all dosages when tested under the conditions described in this study. The findings obtained with EDB and DBCP substantiated the field results reported in table 1.

All fumigants were ineffective in fine textured clay and in soils high in organic matter. Goring (1957) reported that in clay soils the pore space could, under many conditions, be sufficiently small to restrict the molecular diffusion of the fumigant. He reported that reliable soil fumigation was difficult when the organic matter was high because fumigants reacted with soil organic residues.

There appeared to be some relationship between the vapor pressure of the fumigant and its effectiveness in controlling symphylids. For example, the most effective fumigants, methyl bromide and Telone, support vapor pressures of 1670 and 36.0 mg. of Hg, respectively, but ineffective fumigants such as EDB and DBCP have vapor pressures of 15.0 and 0.9 mg. of Hg, respectively. Results of laboratory experiments now in progress support this opinion. Preliminary work conducted to determine basic toxicities of different fumigants indicated that all fumigants were highly toxic to symphylids when released in closed cylinders where diffusion was not a limiting factor. It should be remembered that the symphylid is capable of swift movements in contrast to the sedentary habits of parasitic nematodes, and it may find safety in the lower soil levels before the slow vaporizing fumigants such as EDB and DBCP formed lethal concentrations. It seems probable that the wide difference in the initial rates of vaporization, especially between DBCP, EDB, and Telone, is an important factor in the success or failure of these compounds in controlling symphylids. Soil temperature may therefore be one of the most important factors in determining time of treatments because a change in temperature can affect the diffusion and action of a fumigant in many ways. An increase in the soil temperature would increase the vapor pressure of the fumigant so that the symphylid would be less likely to escape before a lethal concentration was formed.

It is probable that the fumigants would be more effective under the conditions in which cages were employed than under field conditions, even though every effort was made to simulate normal soil compaction. It seems likely that when the soil was replaced after the cages containing symphylids were placed in position (fig. 1), compaction was not as firm as soil in the surrounding undisturbed area. In which case, the fumigant, seeking the line of least resistance, would penetrate to greater depths in the soil contained in the excavation. Furthermore, the effects of the fumigant would be enhanced because the caged symphylids had no opportunity to escape to the lower soil levels. Although this method may be in error in estimating depth of penetration, it provided an accurate method for a comparative evaluation of different fumigants in the control of symphylids. It also proved to be the best method for determining optimum dosages for the control of this pest in the field.

Tables 4 and 5 give the results obtained with Telone and indicated that fumigation with this material greatly reduced the number of symphylids in the growing season following treatment. However, it appeared that sufficient symphylids survived so that additional treatments would be necessary. The findings reported in table 5 showed that there was no apparent difference between treated and untreated plots 11 months after treatment. Since large-sized plots were treated, it was unlikely that reinfestation from untreated areas was responsible for the reinfestation. Lower dosages applied annually probably would be more beneficial in reducing the total population than higher dosages applied at infrequent intervals.

REFERENCES CITED

Essig, E. O. 1926. Paradichlorobenzene as a soil fumigant. California Agric. Expt. Sta. Bull. **411**: 1–20.

Goring, C. A. I. 1957. Factors influencing diffusion and nematode control by soil fumigants. ACD Information Bulletin **110**, The Dow Chemical Company.

Howitt, A. J., J. S. Waterhouse, and R. M. Bullock. 1959. The utility of field tests for evaluating insecticides against the garden symphylid. Jour. Econ. Ent. **52**(3): 666–72.

Morrison, H. E. 1954. A progress report on symphylid research. Ann. Rept. Oregon State Hort. Soc. **46**: 125–9.

Morrison, H. E., and Milton Savos. 1957. 1956 Symphylid investigations. 48th Ann. Rept. Oregon Hort. Soc.

Rasmussen, L. W. 1956. Evaluation of selective weed control in wheat. Weeds **4**(1): 15–21.

Riley, H. K. 1928. A method of volatizing carbon disulfide for soil fumigation. Jour. Econ. Ent. **21**(6): 923–33.

A Modified Microburet for Topical Application of Insecticides to the Onion Maggot, *Hylemya antiqua* (Meig.)[1]

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ABSTRACT

The toxicities of 15 insecticides to the onion maggot, *Hylemya antiqua* (Meig.), were determined by topical application. A modified microburet was used to apply the insecticide solutions to the insect. Toxicity data are recorded as micrograms insecticide per gram of fly. Methyl parathion, dimethoate and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) were the most toxic to the onion fly. Heptachlor, dieldrin, endrin, and DDT were the least toxic.

In topical application of insecticides to individual insects accuracy and ease of operation are most important. Topical application is usually accomplished by treating individual insects with small volumes of insecticide solutions. Trevan (1922) suggested that small droplets of fluids could be produced by using the pitched thread of a machinist's micrometer to drive the plunger of a small syringe. After Trevan developed the micrometer-syringe, topical application became widely employed in insect toxicology, and most of the topical application apparatus in use today embodies Trevan's principle (Metcalf, 1958).

The apparatus used for topical application in the entomology department at Michigan State University is a syringe microburet, made by the Micro-Metric Instrument Co. of Cleveland, Ohio. The syringe microburet (fig. 1) employs a thumbscrew-operated shaft which advances the stem of the direct-dial micrometer gauge. This stem, which is carried through the gauge, advances the plunger of the syringe and delivers the insecticide solution through the syringe. The syringe used in the work reported here delivers a volume of 0.2 microliter of solution for each unit of displacement on the dial. Syringes of other sizes especially calibrated for use with this instrument are available from the manufacturer. The manufacturer also supplies glass buret tips which can be attached to the syringes. In order to deliver small volumes of insecticide solutions accurately, it has been necessary to replace the buret tips by 26-gauge hypodermic needles polished at the tips with a fine abrasive stone and bent at 90° angles.

The advantage of the modified microburet is threefold: 1) Most important, volume deliveries are made quickly and accurately, and they are read on a direct-reading dial gauge. 2) The syringes fit interchangeably into a V-block at the delivery end of the buret stand. 3) Storage facilities for the filled syringes are provided on the buret stand.

In the past year the modified syringe-microburet has been used in connection with onion maggot (*Hylemya antiqua* (Meig.)) studies at this institution. The increasing failure of the chlorinated hydrocarbon insecticides to control this insect (Guyer & Wells 1959) has suggested the development of resistance and has prompted the laboratory evaluation of various materials.

MATERIALS AND METHODS—Experiments were conducted in 1958 on the toxicity of 15 chlorinated hydrocarbon and organic phosphate insecticides to the adults of the onion maggot. All insecticides tested were of technical grade with the exception of methyl parathion, heptachlor, dieldrin, and Guthion® (*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate) which were 80%, 72%, 85%, and 16.7% pure respectively.

The flies used in these experiments were obtained from pupae which had been collected from the field and stored at 38° F. When flies were needed for testing, pupae were transferred to a constant-temperature cabinet at 77° F. and adults allowed to emerge. Flies 2 to 5 days old were used for testing. The adult flies were fed honey and brewers' yeast. The food was prepared on a slice of bread in a petri dish. Water also was added to the petri dish.

The LD50 of each insecticide was determined by applying the toxicant in minute droplets to the mesonota of individual insects. The toxicant was dissolved in acetone and applied by the apparatus described above. One microliter of solution was applied to each fly after the insect had been anesthetized with carbon dioxide. At least four dose levels of each insecticide were used to determine the LD50. Four to five replicates of 10 flies each were used at each dose level. After treatment the flies were held in pint-size ice cream cartons and transferred to a constant temperature cabinet at 77° F. Food and water were supplied to the treated insects. Mortality counts were made 24 hours after treatment.

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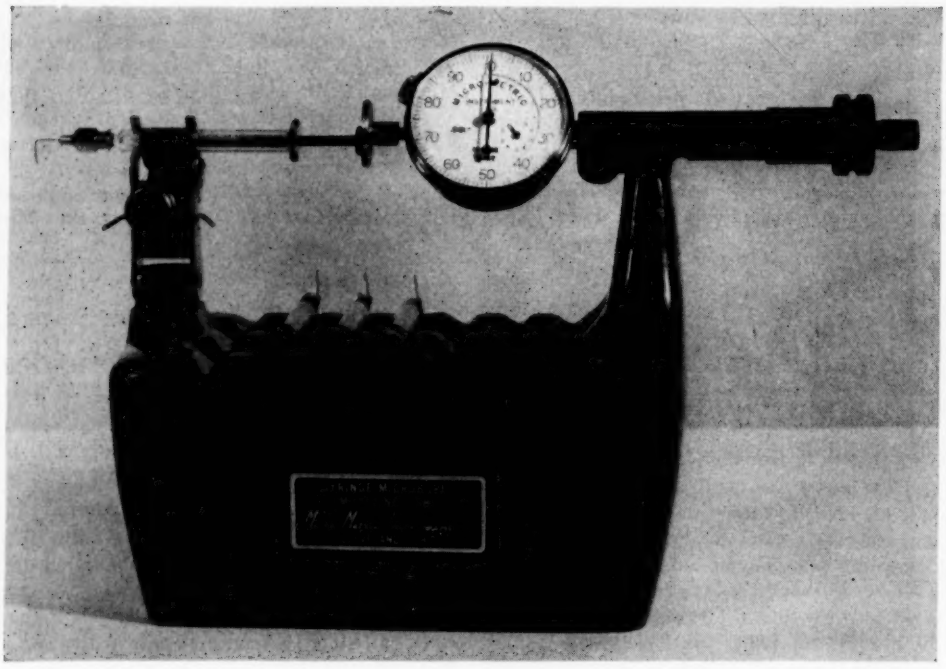

FIG. 1.—The modified microburet.

The criterion for mortality was the inability of the insect to show active locomotion. Thirty flies were weighed to determine the average weight of an onion fly. The LD50 in terms of micrograms of insecticide per gram of fly was determined by the probit analysis method (Finney 1952).

Table 1.—Toxicities of 15 insecticides as determined by topical application to the onion fly.

INSECTICIDE	LD50 IN MICROGRAMS PER GM. FLY	95% CONFIDENCE LIMITS	SLOPE OF LOG. DOSE PROBIT LINE
Methyl parathion	1.30	0.93– 1.80	3.45
Dimethoate	1.32	0.71– 2.44	2.79
Diazinon	1.35	0.67– 2.71	4.94
Parathion	2.09	1.50– 2.90	5.78
Bayer 21/199 (Co-ral®)[a]	2.24	1.74– 2.88	3.82
Am. Cyanamid 18133[b]	2.24	1.24– 4.05	4.76
Trithion	5.72	4.73– 6.90	6.05
Am. Cyanamid 18706[c]	6.92	5.70– 8.40	5.95
Thimet[d]	7.16	4.38–11.40	3.30
Ethion	7.41	6.05– 9.08	6.01
DDT	11.17	7.18–17.38	4.53
Guthion[e]	21.93	16.14–29.93	6.41
Endrin	37.93	24.43–58.88	2.98
Dieldrin	542.00	328– 895	2.28
Heptachlor	2,979.00	2518– 3524	6.42

[a] *O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate.
[b] *O,O*-diethyl *O*-2-pyrazinyl phosphorothioate.
[c] *O,O*-dimethyl-*S*-(*N*-ethylcarbamoylmethyl) phosphorodithioate).
[d] *O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate.
[e] The purity of this material was only 16.7%. See text.

RESULTS—The LD50 in micrograms of toxicant per gram of fly, 95% confidence limits, and the slopes of the log. dose-probit lines for the 15 insecticides tested are presented in table 1. The most effective insecticides against the onion maggot were methyl parathion, dimethoate and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate). The least effective were the four chlorinated hydrocarbon insecticides used. It is to be noted that the sample of Guthion used only was 16.7% pure and was therefore not comparable to the samples of other materials. By calculation, it may be estimated that a sample containing 83.5% Guthion (5 times as pure as used in the laboratory) would have an LD50 of 4.39 micrograms per gram of fly, and would be comparable to Trithion® (*S*-(p-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) in toxicity.

REFERENCES CITED

Finney, D. J. 1952. Probit analysis. Cambridge Univ. Press. 318 pp.

Guyer, G. E., and A. Wells. 1959. Evaluation of insecticides for control of the chlorinated hydrocarbon resistant onion maggot. Michigan Agric. Expt. Sta. Quart. Bull. 41: 614–23.

Metcalf, R. L. 1958. Methods of topical application and injection. In Methods of Testing Chemicals on Insects, vol. 1. Burgess Publishing Co. Minneapolis, Minnesota, pp. 92–114.

Trevan, J. W. 1922. An apparatus for measuring small quantities of fluids. Lancet. 202: 786.

A Technique for the Maintenance of a Laboratory Colony of *Cimex lectularius* L. on Rabbits[1]

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ABSTRACT

A technique is described for the production of several thousand fifth-instar bed bugs per month. The adult colony was fed on rabbits every other day on Monday, Wednesday, and Friday. Egg repository papers with attached eggs were removed before the colony was fed each Friday. Beginning with Monday of the successive week, the newly hatched bugs on the egg papers were fed every other day. After the young bed bugs had been fed four times they were added to the nymphal colony. In the nymphal colony the bugs were held 10 days in newly added rearing chambers and then fed. After three feeding periods the bugs in the beakers were predominantly fifth-instars.

The bed bug, *Cimex lectularius* L., once a pest of man, is now a valuable bioassay tool. It is easy to confine feeds to repletion on host animals in a matter of minutes, and is large enough to handle easily. The fifth instar has a low natural mortality and is very sensitive to minute amounts of toxicant in the blood of the host.

Several techniques for rearing bed bugs are described in literature. Girault (1910) used small glass vials stoppered with cotton for rearing cages. A narrow strip of paper in each vial served as an egg repository. The bed bugs were fed by placing the open mouth of the rearing cage against the lower forearm of a man.

Janisch (1933) used small glass vials covered with bolting cloth for rearing chambers. Circular pieces of filter paper served as egg repositories. The process of feeding the bed bugs was accomplished by strapping the rearing chambers against the shaved belly of guinea pigs that were held motionless by tying them to a special type of board.

Rendtorff (1938) reared large numbers of the bug in petri dishes containing circular pieces of paper toweling. Newly hatched nymphs were separated into small shell vials. Beakers covered with fine batiste cloth were used as feeding chambers. The insects were fed on guinea pigs that were held immobile in a special type of holder.

Woodbury and Barnhart (1939) reared a colony of bed bugs in large petri dishes containing a piece of blotting paper to absorb excreta and serve as a shelter. A cover for the chamber was made from 60-mesh organdy stretched over and cemented between metal embroidery hoops. These covers were held in place by heavy rubber bands. In egg-laying cages, mimeograph paper served as egg repositories. The eggs were loosened and removed with a stiff brush once a week. All stages were fed weekly on rabbits strapped to wooden racks. The colony was maintained at a temperature of 27° C. and 65% relative humidity.

Davis (1956) reared and maintained bed bugs in wide-mouth specimen jars. A piece of heavy blotting paper in each jar served as an oviposition site. Tops for the jars were made of nylon net that was held in place with open-center metal lids. The bed bugs were fed once a week by inverting the jar so that the nylon-net top was pressed tightly against the shaved sides of rabbits. Between feeding periods the insects were kept in an incubator at a temperature of about 30° C. and 70% relative humidity.

Bed bugs collected locally were colonized and have been reared for several years in the laboratory of the Department of Zoology-Entomology of the Agricultural Experiment Station, Alabama Polytechnic Institute. The technique employed for maintaining a balanced colony that produced several thousand fifth-instar nymphs per month is reported herein.

MATERIALS AND METHODS.—Chambers for feeding bed bugs were fashioned from Pyrex glass cylinders 85 mm. in diameter and 60 mm. high. Half-pint ice cream containers with the bottoms removed were also used. The floors of the chambers consisted of a double layer of nylon hosiery material glued and taped over one end of the cylinders. The open ends of the cylinders were closed by lids from half-pint ice cream containers. The centers of these lids were removed and replaced by hosiery material.

Glass beakers, 250-ml. capacity, were used to hold the bed bugs between feeding periods and for rearing the immature stages. The beakers were kept in metal trays in incubators at 26.7° C. and 26% relative humidity. These rearing chambers contained egg repositories, which consisted of dated 60-mm. disks cut from paper towels.

The inner walls of both the glass feeding and rearing chambers were furbished with either cheesecloth or paper toweling. A clean surface reduced the possibility of the bed bugs climbing the walls.

PROCEDURE.—Sixteen rearing chambers each containing 200 to 300 vigorous adults were maintained for egg production. Maximum egg production was achieved by feeding the adult bed bugs each Monday, Wednesday, and Friday. At each feeding period the adults and egg repositories were transferred to feeding chambers. These feeding chambers were then placed on the shaved abdomen of rabbits that were strapped on their backs in specially constructed wooden stanchions. Heavy rubber bands held the chambers tightly against the rabbits, allowing the bed bugs to feed through the nylon floor of the chamber. Two feeding chambers were placed on each rabbit for 30 minutes at each feeding period.

Following feeding, the egg repositories and any attached bed bugs were transferred from the feeding chambers to the rearing chambers. The feeding chambers were then inverted over a finger bowl into which the weak or dead insects and cast skins dropped and were discarded. The feeding chambers were again inverted over other finger bowls and the viable adults were anesthetized with carbon dioxide. After 5 to 10 seconds the insects relaxed and dropped from the nylon floor of the feeding chamber to the bottom of the finger bowl and were then transferred

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to the rearing chambers. These bed bugs were anesthetized, and the egg repositories were removed weekly before the Friday feeding period. These repositories were divided into two groups and placed in beakers for incubation of the eggs. The adults were then provided fresh paper disks for oviposition.

Shortly after the egg papers were removed the eggs began hatching. The resulting first-instar nymphs were fed each Monday, Wednesday, and Friday to reduce mortality. After four feeding periods, the first-instar nymphs were held without food for 10 days to allow all the bed bugs to molt into second-instar nymphs, after which they were fed at 10-day intervals. Following the third feeding, fifth-instar nymphs and a few adults began to appear. These adult bed bugs were then transferred to the adult holding chambers for egg production. Fifth-instar bed bugs were held for experimental use. Those fifth-instar nymphs not needed for experiments were allowed to feed a fourth time and to molt to the adult stage, and were added to the adult holding chambers for further egg production.

REFERENCES CITED

Davis, N. T. 1956. The morphology and functional anatomy of the male and female reproductive systems of *Cimex lectularius* L. (Heteroptera, Cimicidae). Annals Ent. Soc. America **49**(5): 466–93.

Girault, A. A. 1910. Preliminary studies on the biology of the bed bug, *Cimex lectularius*, Linn. Jour. Econ. Biol. **5**(3): 88–91.

Janisch, E. 1933. Beobachtungen bei der Aufzucht von Bettwanzen. 1. Über das Verhalten von Populationen bei verschiedenen Zuchtbedingungen. Zeitsch. Parasitenk **5**: 460–514.

Rendtorff, R. C. 1938. A method of rearing the bed bug, *Cimex lectularius* L., for studies in toxicology and medical entomology. Jour. Econ. Ent. **31**(6): 781.

Woodbury, E. N., and C. S. Barnhart. 1939. Tests on crawling insects, tentative methods for evaluating liquid household insecticides against the German cockroach and the bed bug. Soap and Sanitary Chem. **15**(9): 93–107, 113.

Field Evaluation of Four Insecticides for the Prevention of Lettuce-Yellows Disease with Known Populations of Viruliferous Six-Spotted Leafhoppers[1]

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ABSTRACT

Artificially reared viruliferous six-spotted leafhoppers, *Macrosteles fascifrons* (Stål), were caged over small areas of lettuce in the field to test the effectiveness of four insecticides (DDT, demeton, malathion, and parathion) applied as emulsion sprays for lettuce-yellows prevention. This leafhopper is the only known vector for the eastern strain of the virus *Chlorogenus callistephi* var. *vulgaris* Holmes, causing lettuce-yellows disease in New York State. Differences were found between sprayed and unsprayed lettuce in the incidence of disease, and in the residual effectiveness of insecticides tested. Differences between insecticides were significant but not readily apparent by mere visual inspection. The use of known populations of viruliferous leafhoppers permitted evaluation of sprays where ordinary small-plot and large-field experiments had failed.

Insecticides are applied to lettuce for the control of the six-spotted leafhopper, *Macrosteles fascifrons* (Stål), in New York State primarily for disease prevention as this insect is the only known vector for the eastern strain of the virus *Chlorogenus callistephi* var. *vulgaris* Holmes, causing lettuce yellows. It is the purpose of this paper to report results from field experiments whereby artificially reared viruliferous six-spotted leafhoppers were caged over small areas of lettuce to test the effectiveness of four insecticides applied as emulsion sprays for lettuce-yellows prevention. In addition, results which deal primarily with the technique of using known populations of leafhoppers for field tests are included since these results led to refinements in the "known population" procedure.

The need for an experimental method which permitted better control over populations of infective leahfhoppers becomes apparent from a brief review of experiences with ordinary small-plot and large-field experiments during the early stages of this study. Results from ordinary small-plot tests conducted by the authors failed to differentiate between insecticidal spray treatments and unsprayed controls in the incidence of lettuce-yellows disease. The projected reasons for insignificant differences were: (1) the sprays failed to kill leafhoppers, and (2) migrating insects from unsprayed control plots and adjoining areas quickly reinfested sprayed lettuce. Data from virus transmission studies reported by Strong & Rawlins (1958a) suggested that unless insecticide residues on lettuce plants were effective in killing leafhoppers quickly, reinfestation of treated small plots might occur soon after application of sprays and the incidence of yellows disease likely would be as high in treated plots as in the untreated controls. Pepper & Haenseler (1939) previously found this to be true. They then applied the same insecticides that had been ineffective in small-plot trials to large plots and reduced yellows significantly on them.

The large-field type of experiment was not entirely satisfactory in the present study, however, for a thorough evaluation of insecticides used for the prevention of lettuce-yellows disease. Practical methods for sampling leafhopper populations in lettuce were unavailable, so both

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insect control and prevention of virus transmission had to be measured by the incidence of disease. This depended upon the presence of infective insects. To make good comparisons between insecticides a high-potential yellows incidence (50% or more) was considered essential, but the average incidence of disease in most lettuce fields was low during the 3-year period of these investigations, presumably owing to a very small proportion of infective leafhoppers in the population. Indeed, indexing of leafhoppers taken from several fields of lettuce and carrots during one season showed infective individuals in such low proportions as 0 to 10% of the numbers tested. Thus, it became obvious that artificial populations must be used in field experiments rather than dependence on the natural incidence of infective insects.

A detailed account follows of field experiments in Oswego County, New York, with known populations of viruliferous six-spotted leafhoppers caged over lettuce to test the effectiveness of insecticides for the prevention of lettuce-yellows disease.

MATERIALS AND METHODS.—Insecticides tested and the pounds of toxicant per gallon of emulsifiable concentrate were: DDT 2, demeton 2, malathion 5, and parathion 2. Dosages of insecticides applied are given as ounces of toxicant per acre per application of spray.

Sprays were applied at 6- to 10-day intervals, depending on weather conditions, with a low-pressure sprayer mounted on a garden-type tractor. The sprayer delivered 100 g.p.a. through two *TeeJet* No. 8002 nozzles per row at a speed of 2 m.p.h. and a pressure of 40 p.s.i. Five 15-inch-wide rows were covered in each swath. Applications began after seedling plants emerged from the soil, when the first true leaf was evident, and extended to completion of an experiment.

Large cages (7′×7′×2′) were placed over lettuce to confine viruliferous leafhoppers to treatment areas. As noted later, smaller cages were used in one experiment. Cage frames were made of wood and covered with nylon tulle or marquisette. Air circulated freely over the plants and preliminary tests indicated little or no effects from insecticide fumes inside cages. Before moving cages from one experimental area to another, each cage was covered with a tarpaulin and disinfested with an aerosol containing pyrethrins.

Six-spotted leafhoppers were produced by the mass rearing methods described by Strong & Rawlins (1957). Viruliferous insects for the first experiment reported in this paper were supplied from greenhouse cultures, but for reasons noted later, insectary procedures were used to provide leafhoppers for other experiments.

Young adult leafhoppers were permitted an infection-feeding period of not less than 3 days on China aster plants which showed definite symptoms of virus (*Chlorogenus callistephi* var. *vulgaris*) infection. They were then transferred to barley and held until ready for use. At least 18 days' incubation of virus in the insects expired from the time of first exposure to diseased China aster plants, before leafhoppers were used to infest caged areas of experimental lettuce. Previous studies by Strong & Rawlins (1958a) indicated that such infection feeding and virus incubation periods would insure the presence of large numbers of infective individuals among viruliferous leafhoppers, thus providing the high-potential incidence of lettuce yellows considered necessary for field tests.

All tests plants were examined for symptoms of lettuce-yellows disease, usually 1 month after exposure to viruliferous leafhoppers. The incidence of diseased plants was the only criterion recorded for the comparison of treatment results.

Additional methods or variations in procedures which are concerned with specific experiments are described where they are pertinent in this report.

EXPLORATORY EXPERIMENT WITH VIRULIFEROUS LEAFHOPPERS CAGED OVER LETTUCE.—If the "known population" procedure was to be used to its best advantage in field tests with insecticides, a comprehensive exploratory study was essential. Not only was it desirable to compare the initial and residual effectiveness of insecticides in preventing lettuce yellows, but phases of the problem concerning refinements in the technique employing known populations of leafhoppers in the field were equally important.

Procedure.—Treatments were replicated three times in a split split plot design. Replications were made at two locations, two on one farm and one on another. Whole plot treatments (A in table 2) were the number of spray applications made before exposing lettuce to viruliferous leafhoppers, namely: 1, 3, and 5 applications. Split plot treatments (B in table 2) were the intervals from the time of spraying until viruliferous insects were caged over plants—2 hours, 3 days, 6 days, and 10 days, respectively. Split split plot treatments (C in table 2) were the sprays applied. Insecticides used and dosages per acre per application were: DDT 8 ozs., demeton 3 ozs., malathion 20 ozs., and parathion 4 ozs.

Cages were placed over lettuce after completing the first application of sprays. These were infested within 2 hours after spraying with 50 viruliferous leafhoppers per cage. Leafhoppers were allowed a test-feeding period of 3 days before disinfestation and removal of cages. The same test-feeding period and number of leafhoppers per cage were used for the exposure of test lettuce to viruliferous insects at intervals of 3, 6, and 10 days following the spray application. The procedure described for infesting lettuce with viruliferous leafhoppers was repeated for whole plots receiving three and five applications of insecticides before exposure to test insects.

Lettuce was not thinned in whole plots infested with viruliferous leafhoppers after 1 spray application. In treatment areas receiving 3 and 5 applications of spray before infestation, plants were spaced as normally left when blocking lettuce, about 30 plants per split split plot.

Yellows-incidence counts were completed 1 month following initial exposure of lettuce to viruliferous leafhoppers in all plots except those receiving five spray applications before exposure to insects. In the latter, some counts were made 15 days after the last application because of the rapid maturity and deterioration of lettuce. All plants were inspected for disease symptoms.

Results and Discussion.—Results (in nearest whole numbers) are presented in table 1 as summarized for the entire experiment. Tabulated data were analyzed by the usual analysis of variance procedures for split split plot designs (Federer 1955), and the analysis is given in table 2. Percentages of lettuce yellows were transformed to angles corresponding to percentages before analysis ac-

Table 1.—Mean percentages of plants with symptoms of lettuce-yellows disease as summarized for the exploratory field experiment with caged viruliferous six-spotted leafhoppers.

Insecticide Treatment	Toxicant per Acre per Application (oz.)	Time from Spraying to Cage Infestation: 2 Hrs.	3 Days	6 Days	10 Days	Mean
		Per Cent Lettuce Yellows				
DDT	8	27	34	42	40	37
Demeton	3	18	34	38	44	33
Malathion	20	14	31	43	28	29
Parathion	4	21	28	47	43	35
Unsprayed	0	30	36	44	37	37

cording to Snedecor's (1946) suggestions.

The variation attributed to the intervals from the time of spraying until viruliferous leafhoppers were caged over lettuce (B—Split plots in table 2) was tested against the error variance by means of the F test and found to be significant at the 1% level, and the variation attributed to insecticide spray treatments (C—Split split plots in table 2), including unsprayed controls, was found to be significant at the 5% level. The interaction between the time viruliferous leafhoppers were caged over lettuce plants after application of sprays and insecticide treatments (B×C in table 2) was found to be significant at the 5% level. As seen in table 2, no other variables or interactions were significant.

Considering the means for the entire experiment in table 1, differences are not readily apparent between insecticide treatments by mere visual inspection. But for the 2-hour interval from application of sprays to infestation of caged lettuce with viruliferous leafhoppers, differences between insecticides are indicated. It is of interest to note that extreme differences are not found in table 1 between insecticides or between sprayed lettuce and unsprayed controls.

The incidence of lettuce yellows in unsprayed control plots was lower than expected, based on the studies of Strong & Rawlins (1958a), and did not reach the level desired for field experiments. Tests determining the minimum infection feeding and virus incubation periods necessary for maximum percentages of infective leafhoppers, however, were conducted by Strong & Rawlins in a greenhouse during the winter with a temperature range of 70 to 90° F. Viruliferous leafhoppers were produced for this experiment during the summer when greenhouse temperatures became very high during the day. Since Kunkel (1937, 1941a,b) has found that the causitive virus could be inactivated by heat, it appeared logical to produce viruliferous leafhoppers and virus source plants for subsequent experiments in insectaries near the summer field operations where temperatures could be maintained about the same as those found under natural conditions.

Differences between whole plots were not shown to be significant in the analysis of variance presented in table 2, but, from the viewpoint of efficient field procedure, exposure of lettuce to viruliferous leafhoppers after the third application of spray appeared to be the most desirable in experiments of this kind. The third spray is usually applied immediately after blocking, when plants are about 1½ inches tall. Then lettuce can be thinned to the desired stand. It grows rapidly during the next 30 days and disease symptoms are recognized easily.

Table 2.—Analysis of variance of angles corresponding to percentages of lettuce yellows in the exploratory field experiment with caged viruliferous six-spotted leafhoppers.

Sources of Variation	Degrees of Freedom	Mean Square
Replicates	2	3,434.71
A—Whole plots	2	9,270.33
Error (a)	4	1,985.62
B—Split plots	3	1,756.62**
A×B	6	400.76
Error (b)	18	293.28
C—Split split plots	4	116.02*
A×C	8	34.85
B×C	12	80.27*
A×B×C	24	49.17
Error (c)	96	36.87
Total	179	

Exposure of Lettuce to Varying Numbers of Viruliferous Leafhoppers.—From the results obtained in the preceding experiment, definite procedures could be formulated for the efficient use of known populations of viruliferous leafhoppers in the field, but one important question remained: the approximate population of infective leafhoppers needed to insure a high incidence of lettuce yellows in field tests. This was determined by caging varying numbers of leafhoppers on lettuce as described below.

Procedure.—Treatments were replicated eight times in a split plot design. Whole plot treatments were the insecticide sprays, including unsprayed controls, and split plot treatments were the insect/plant ratios given in table 3. Twenty cages were used to confine viruliferous leafhoppers over lettuce. Ten were smaller (6′×2′×2′) than those previously described. Although cages were of two sizes, each insect-plant-ratio treatment had both an equal number of times. Lettuce was thinned to 20 plants per plot, regardless of the size cage used.

The desired numbers of viruliferous leafhoppers per plot were introduced into cages within 1 hour following the third application of sprays. These were permitted a 3-day test-feeding period. All plants were examined for disease symptoms 1 month after initial exposure to viruliferous insects.

Results and Discussion.—A higher and more desirable incidence of yellows disease resulted from the use of two insects per plant than from one per plant, but differences are apparent in table 3 between sprayed lettuce and unsprayed controls for both insect/plant treatments (one and two leafhoppers per plant).

Immediate and Residual Effectiveness of Insecticides.—The final experiment in this study was conducted explicitly for the purpose of comparing immediate and residual effectiveness of four insecticides in preventing lettuce-yellows disease.

Procedure.—Treatments were replicated six times in a split plot design. Whole plot treatments were the in-

Table 3.—Mean percentages of yellows in lettuce exposed to varying numbers of viruliferous leafhoppers immediately after spraying.

Insecticide Treatment	Toxicant per Acre per Application (oz.)	Insect/Plant Ratio: 2/ Plant	1/ Plant	1/5 Plants	1/10 Plants
		Per Cent Lettuce Yellows			
DDT	12	40	24	8	13
Demeton	6	33	22	13	8
Malathion	20	22	17	9	3
Parathion	6	28	19	4	6
Unsprayed	0	53	40	18	13

Table 4.—Mean percentages of yellows disease in lettuce exposed to two viruliferous leafhoppers per plant 1 hour and 3 days after spraying.

Insecticide Treatment	Toxicant per Acre per Application (oz.)	Time from Spraying to Cage Infestation: 1 Hour	3 Days
		Per Cent Lettuce Yellows	
DDT	12	34	64
Demeton	6	42	57
Malathion	20	32	71
Parathion	6	45	72
Unsprayed	0	89	78

secticide sprays listed in table 4. Split plot treatments were the intervals from spraying to exposure of caged plants to viruliferous leafhoppers—1 hour and 3 days after spraying.

Lettuce was thinned to 25 plants per plot. Fifty viruliferous leafhoppers, 2 per plant, were placed in each cage within 1 hour following application of sprays. Insects were allowed an infection-feeding period of 3 days before cages were disinfested and moved. The same procedure was followed for the exposure of test lettuce 3 days after application of sprays.

Results and Discussion.—The incidence of lettuce-yellows disease was recorded 1 month following exposure of lettuce to viruliferous leafhoppers. Every plant was inspected for disease symptoms, and 20 to 25 plants remained in each plot at this time. The results are summarized in table 4.

Obvious differences between sprayed and unsprayed lettuce in the incidence of lettuce-yellows disease are found in the data presented in table 4 where lettuce was exposed to leafhoppers within 1 hour after application of sprays. Less obvious differences, however, are found between insecticides. Little residual value of insecticide treatments was indicated.

GENERAL COMMENTS.—The use of known populations of viruliferous six-spotted leafhoppers to test the value of insecticides in preventing lettuce-yellows disease was successful in differentiating between sprayed and unsprayed lettuce in the incidence of disease, and in the residual effectiveness of insecticides tested. Differences between insecticides were not as evident as had been anticipated, and the degree of protection from yellows infection would most likely not be satisfactory to lettuce growers. It should be remembered, however, that the controlled populations of viruliferous leafhoppers were intentionally severe.

It is apparent that satisfactory interpretation of results obtained with known populations of viruliferous leafhoppers is dependent on a knowledge of the toxicity of sprays to leafhoppers. For the four insecticides tested, this information was provided in time-mortality tests reported previously by Strong & Rawlins (1958b).

REFERENCES CITED

Federer, Walter T. 1955. *Experimental Design.* Macmillan, New York.

Kunkel, L. O. 1937. Effect of heat on ability of *Cicadula sexnotata* (Fall.) to transmit aster yellows. Amer. Jour. Bot. **24:** 316–27.

Kunkel, L. O. 1941a. Heat cure of aster yellows in periwinkles. Amer. Jour. Bot. **28:** 761–9.

Kunkel, L. O. 1941b. Cure of aster yellows by heat treatments. (Abstract) Phytopath. **31:** 14.

Pepper, Bailey B., and C. M. Haenseler. 1939. Pyrethrum and derris as a control for the six-spotted leafhopper, a vector of lettuce yellows. Jour. Econ. Ent. **32:** 291–6.

Snedecor, George W. 1946. *Statistical Methods.* Iowa State College Press, Ames.

Strong, R. G., and W. A. Rawlins. 1957. Mass rearing of six-spotted leafhoppers. Jour. Econ. Ent. **50:** 76–9.

Strong, R. G., and W. A. Rawlins. 1958a. Aster-yellows virus infection and transmission by *Macrosteles fascifrons* (Stål) with reference to lettuce-yellows prevention. Jour. Econ. Ent. **51:** 512–5.

Strong, R. G., and W. A. Rawlins. 1958b. Evaluation of four insecticides for the control of six-spotted leafhoppers on lettuce. Jour. Econ. Ent. **51:** 903–5.

Walnut Serpentine Leaf Miner, *Nepticula juglandifoliella* Clem., a Pest of English Walnut[1]

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Abstract

A destructive population of the walnut serpentine leaf miner, *Nepticula juglandifoliella* Clem., was encountered infesting English walnut in a localized area in the Salinas Valley near King City, California. This is the first report of this insect's causing serious damage to walnut in this State. Two hundred of 600 acres were either severely or seriously damaged.

It is believed that under ordinary conditions the pest is held in check by natural enemies and that treatments of demeton applied to control the walnut aphid may have interfered with effective parasitism.

Parasites were very abundant and the following species of the family Eulophidae were reared from infested leaves: *Closterocerus utahensis californicus* Girault, *Zagrammosoma nigrolineatum* Cwfd., and *Solenotus begini* (Ashm.).

The walnut serpentine leaf miner, *Nepticula juglandifoliella* Clem., has developed into a serious pest of English walnut in a localized area in the Salinas Valley near King City, California. The insect is not new to the State as Barrett (1932) reported it on both black and English walnut. It is a species that occurs in the eastern as well as western United States. In the former area Braun (1917) listed the hosts as hickory, walnut, and butternut. In a letter H. H. Keifer, Systematic Entomologist, California State Department of Agriculture, stated that in California the walnut serpentine leaf miner occurs commonly on native black walnut, but he was somewhat surprised to hear of it as a pest of English walnut. Although the insect is rather widespread in California, no serious outbreak on walnut had been encountered until attention was called to the situation near King City. In that area the outbreak occurred in a region where 600 acres of walnuts are grown. The leaf miner had been observed by the grower as causing some injury for some years, but damage did not reach alarming proportions until 1958.

Injury and Biology.—The infestation was first examined on July 30, 1958. At that time about 100 acres of the 600 were seriously infested. The intensity of the attack varied considerably in the area where damage was being done. In the region of most severe injury some defoliation had already occurred and much of the foliage remaining on the trees was heavily damaged. Many leaflets contained 150 or more mines. As the intensity of attack increases there is a tendency for the heavily mined areas of a leaf to dry up and turn brown. When this condition occurs the leaflets start to curl and in the final stage injury is so great that the leaves drop from the tree. In figure 1 is shown a heavily mined leaflet illustrating the situation that exists at about the time patches of the leaflet start to dry up and turn brown. In figure 2 is shown a much enlarged mine in which the fecal or frass track left by the larva is clearly seen.

When the orchard was visited on July 30, the adult leaf miners were present in swarms. They were especially active in the shade beneath the canopy of the trees or in areas sheltered from the wind. Many adults in copulation were observed on the underside of the leaves. The adults moved with great rapidity over and under the leaves. They were readily disturbed and flew up in large numbers but quickly settled and usually moved to the undersurface of the leaves. The eggs which are oval, smooth, flat, and shiny are laid on the undersides of the leaflets. Almost all the eggs are deposited next to a vein. In hatching the larvae bore directly through the underside of the egg and penetrate the leaf tissue. Larval development occurs just under the upper epidermis of the leaf. At first the mine is tiny, but it increases in diameter as the larva grows. The developing larva can be seen feeding in the mine, which it enlarges by moving its head from side to side while feeding. Upon reaching maturity the larva cuts an exit hole at or near the end of the mine through the upper epidermis of the leaf. It then drops to the ground to pupate. The piece cut out to allow the larva to escape is left attached at the base and appears somewhat like a flap.

Fig. 1.—A walnut leaflet heavily infested by the walnut serpentine leaf miner, *Nepticula juglandifoliella* Clem.

On July 30, many mines were encountered from which the larvae had emerged. The large number gave evidence that the insect had probably passed through no less than two generations previous to the time of the survey. Further, there was some evidence that the pest might be rather even brooded. This was indicated by the fact that no unhatched eggs were observed, and by the swarms of

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adults, many of which were in copulation. There were many miners in the leaves and counts showed that the majority of the larvae in these mines were parasitized. In some localities parasitism exceeded 90%. The presence of swarms of adult moths indicated, however, that even this high degree of parasitism was insufficient to protect the trees adequately in areas of heaviest infestation. Thus 100 of the 600 acres were severely damaged at harvest, 100 additional acres were seriously injured, and the rest escaped damage of an economic nature.

PARASITES.—The following parasites of the family Eulophidae were reared from infested leaves: *Closterocerus utahensis californicus* Girault, *Zagrammosoma nigrolineatum* Cwfd., and *Solenotus begini* (Ashm.). Under ordinary conditions parasites are probably very important in suppressing the leaf miner population. In the present case there is little doubt but that parasites were responsible at least in part for preventing serious damage in much of the area. On September 19, a survey conducted in one of the orchards on the periphery of the heavily infested area showed that only 3 out of 296 larvae examined escaped parasitism. The infestation in this orchard was effectively arrested.

Some difficulty was experienced in following the exact situation with regard to parasitism that existed in the region of severe damage. Although numerous surveys were made it was hard to establish a definite trend in the amount of parasitism that was occurring. There were many complicating factors. One of these was the very high mortality of the host, probably the result of competition for space. The exact manner in which this competition interfered with parasitism is not known, but under these conditions parasitism dropped to a very low level. However, out of 810 individuals examined on the final survey conducted on November 20, 2 were alive, 484 were dead, 192 were parasitized, and parasites had emerged from 132. The season ended with a favorable relation between the host and parasite populations.

FIG. 2.—Mine made in walnut leaf by the walnut serpentine leaf miner, *Nepticula juglandifoliella* Clem. ×12

Table 1.—Seasonal distribution of parasites attacking the walnut serpentine leaf miner.

SPECIES	ADULT PARASITES EXPRESSED AS PERCENTAGE OF TOTAL INDIVIDUALS THAT EMERGED FROM LEAF SAMPLES COLLECTED ON:				
	July 30	Sept. 19	Oct. 1	Oct. 29	Nov. 1
Closterocerus utahensis californicus	—	2.7	—	69.3	94.0
Solenotus begini	—	3.1	1.0	7.7	—
Zagrammosoma nigrolineatum	100.0	94.2	99.0	23.0	6.0

On five occasions during the summer and fall, parasites were reared from infested foliage. The parasites were separated according to species, and the per cent of each species occurring in each of the collections is given in table 1. An interesting seasonal distribution in the species occurred; *Zagrammosoma nigrolineatum* was the predominant one during the first part of the period while *Closterocerus utahensis californicus* was the principal species late in the season.

DISCUSSION.—The leaf miner was found infesting foliage of apricot in an orchard adjacent to a heavily attacked walnut grove. The infestation in apricot was light and probably resulted from the intense population pressure developed in the walnut orchard. Larval growth on apricot appeared normal. Mines were observed in which larvae had completed development and others contained live larvae.

One can only speculate as to the probable reason for the severe outbreak of the leaf miner on walnut. From the information obtained it is believed that normally the insect is held in check by natural enemies and that the increase of the insect to a destructive level might be associated with some interference with parasitism. For the past few years the orchards involved have received spring treatments of demeton to control the walnut aphid, and these treatments may be responsible for the increase in the leaf miner problem. It is possible that demeton has no adverse effect upon the leaf miner larvae, but that there may be sufficient demeton ingested to be highly toxic to the tiny parasite larvae at the time they hatch from the eggs and begin to feed on the host. During the coming season an effort will be made to determine whether such a relation actually exists.

ACKNOWLEDGMENTS.—We should like to express our appreciation to Arnold Frew and Arthur Brown, of the California Orchard Company of King City, for their excellent cooperation in the conduct of this investigation. Thanks are also due to B. D. Burks of the Insect Identification and Parasite Introduction Laboratories, U. S. Department of Agriculture, for the identification of the parasites. For field and laboratory assistance we

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REFERENCES CITED

Barrett, R. E. 1932. An annotated list of the insects and arachnids affecting the various species of walnuts or members of the genus *Juglans* Linn. Univ. California Publ. Ent. **5**(15): 275–309.

Braun, Annette F. 1917. Nepticulidae of north America. Trans. Amer. Ent. Soc. **43**: 155–209.

The Metabolism of Co-Ral (Bayer 21/199) by Tissues of the House Fly, Cattle Grub, Ox, Rat, and Mouse[1]

R. D. O'BRIEN and L. S. WOLFE[2]

ABSTRACT

In mammals, the liver is primarily responsible for Co-ral metabolism. In the mouse, which is susceptible to Co-ral® (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothionate) the compound is activated, and there are no degrading systems. In the ox and rat, which are resistant to Co-ral, the compound is degraded. It has been shown for the ox that both an activating and a degrading system are in the liver, but the latter is more potent. In the house fly (*Musca domestica* L.), and cattle grub (*Hypoderma bovis* (L.)), which are susceptible to Co-ral, there is an activating but no degrading system. Activation by the cattle grub is associated with fat body and gut. The results account for the selective toxicity of Co-ral, and possibly for the fact that only dermal treatment controls cattle grubs in the ox.

Bayer 21/199 (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothionate), referred to in this paper by its registered trademark name Co-ral®, has been used successfully for systemic control of cattle grubs (Smith & Richards 1954, Roth & Eddy 1955, Chemagro 1957). It is therefore presumably of low toxicity to the ox and high toxicity to the grub. However, although it is effective against the grub when applied dermally to the cattle, it is ineffective when applied orally (MacDougall 1958).

The metabolism in the rat, goat, and ox has been studied by Krueger *et al.* (1958), who show that degradation can occur by phosphatase action on the ethoxy as well as on the linkage. No examination was made specifically for the oxidized form of Co-ral but (if present) it was extracted with Co-ral. Lindquist *et al.* (1958) have shown that the rat rapidly excretes Co-ral as polar compounds.

The intention of the present study was to establish whether the selective toxicity of Co-ral could be accounted for on the basis of differences in the balance of toxifying and detoxifying systems in various animals, as has already been shown for malathion (March *et al.* 1956, O'Brien 1957a) and acethion (O'Brien *et al.* 1958).

METHODS.—Tissues were prepared either by slicing with an automatic tissue slicer (H. Mickle Co., Gormshall, England); mincing in a household meat-mincer in the cold-room; or homogenizing in an iced high-speed homogenizer (Servall Omnimixer).

The tissue preparations were incubated in 25 or 50 ml. of Krebs-Henseleit buffer (Krebs & Henseleit 1933) containing also 0.2% glucose. In the first method, the incubation mixture was shaken in an open beaker on the platform of a wrist-action shaker, while wet air was bubbled through. In the later studies, detergent (Triton X-100) was added to 0.1% concentration, and the beakers were shaken at about 120 strokes per minute on a reciprocal shaker, without air-bubbling. In both cases, a 2-ml. sample was withdrawn, then 1 ml. of 10^{-2} M Co-ral in ethanol was added, and further samples were withdrawn at timed intervals. All samples were frozen rapidly in vials held in acetone-dry ice.

Later the samples were thawed and 0.5 ml. was added to Warburg flasks containing 0.33 ml. of human plasma, 0.25 ml. of $NaHCO_3$ 1.25%, 1 ml. of 0.033 M phosphate buffer pH 7.4 and 0.42 ml. of water. The side-arms contained 0.2 ml. of 5% acetyl choline bromide. The cholinesterase was assayed manometrically in the usual way at 25° C., the substrate being tipped in precisely 90 minutes after addition of the sample. In the cases where the tissue used was sliced or minced, the samples for cholinesterase assay were pipetted up through a plug of glass wool. In the cases reported below of "no effect of tissue upon Co-ral," the experimental observation was a horizontal line for the plot of "per cent cholinesterase inhibition" against "time." Such curves have not usually been reproduced in this paper.

The animals used were as follows; mice: 25 to 30 gm. SW females, Taconic Farms, Germantown, New York; rats: 200 to 300 gm. females, Holtzman Company, Madison, Wisconsin; ox; steer; house fly, *Musca domestica* L., reared in this laboratory, 3 to 4 days, mixed sexes; cattle grubs, 3rd instar *Hypoderma bovis* (L.), removed from steers at slaughter and stored at 4° C., used within 3 days, alive as judged by contractility.

RESULTS.—*Toxicity.*—The LD_{50} of Co-ral to the house fly was 1.5 mg./kg., to the mouse (intraperitoneal) 23.5 mg./kg. (compare 22.5 mg./kg. Chemagro 1957) and to the rat (intraperitoneal) 150 mg./kg. (Chemagro 1957). Taken with the effectiveness of Co-ral in cattle-grub control, we may say that house fly, cattle grub, and mouse are susceptible, rat and cow are not.

Solubility and variability.—The poor solubility characteristics of this compound caused some difficulties. When as little as 0.1 ml. of a 10^{-2} ethanolic solution was added

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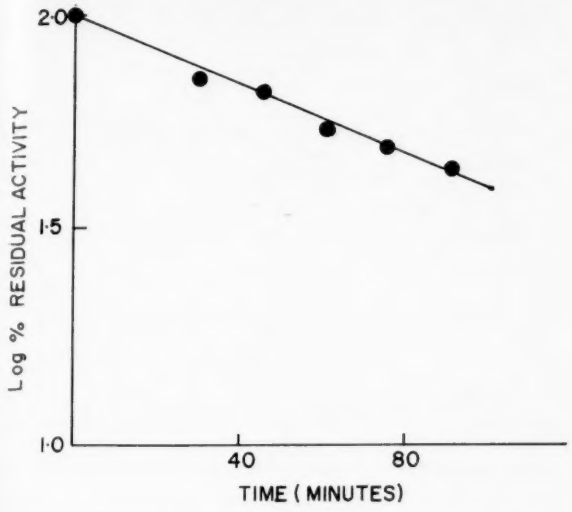

Fig. 1.—Inhibition of plasma cholinesterase by Co-ral. Time is that of incubation period with cholinesterase.

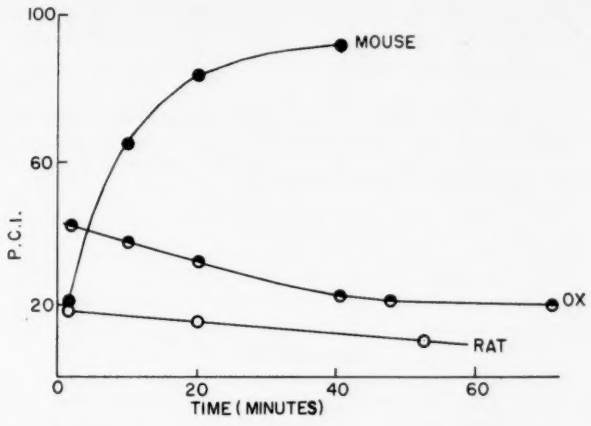

Fig. 2.—Effect of liver slices upon Co-ral solution. Time is period of contact between slices and Co-ral. P.C.I. = per cent cholinesterase inhibition.

to 10 ml. of water, an opalescent colloidal solution resulted and in a few hours much of the Co-ral had crystallized out. Attempts to inhibit this crystallization were made, using gelatin, blood albumen, plasma, and detergents. Eventually 0.1% Triton X-100 was selected as preventing the initial opalescence and inhibiting crystallization, while giving little biochemical interference.

As will be seen in the accompanying graphs a considerable variability in the anticholinesterase activity of the intial samples was observed, a phenomenon not found in previous studies with malathion or acethion. Krueger *et al.* (1958) found unusual difficulty in extracting tracer-labeled Co-ral into chloroform from numerous protein-containing solutions; no change in case of extraction was found after 30 minutes. Presumably proteins bind the Co-ral, and our variablity is caused by differences in the extent of binding with the various preparations used.

Anticholinesterase Activity.—The variation of inhibition of human plasma cholinesterase with Co-ral concentration was studied: 50% inhibition was given by 6×10^{-5} M Co-ral (incubation time 90 min.). The variation of the logarithm of inhibition with time was linear (fig. 1) and therefore conformed with pseudo-first order kinetics, giving a bimolecular rate constant of 1.24×10^{-2} l. moles^{-1} min.$^{-1}$ (Aldridge 1950). This gives empirical support to the use of the plasma for assaying Co-ral, and indicates that there is no Co-ral degrading enzyme in the plasma.

The oxygen analogue of Co-ral (*O,O*-diethyl *O*-(3-chloro-4-methyl-7-coumarinyl) phosphate), termed "coroxon" below, was prepared by bromine oxidation of a solution made by adding 0.5 ml. of Co-ral, 10^{-2} M in ethanol, to 6.5 ml. of water, using the method employed for malathion by Murphy & Dubois (1957). This preparation of coroxon inhibited cholinesterase by 50% at 5×10^{-8} M. In view of the large difference in anticholinesterase activity of Co-ral and coroxon, any production of the latter when Co-ral was added to tissue preparations could be followed readily by sampling for anticholinesterase activity.

Metabolism by Mammalian Tissues.—It has been established for all phosphorothionates studied that conversion to the potent anticholinesterase phosphate analogues ("activation") can be carried out in the whole animal or by liver slices, but very little in tissue homogenates, unless the latter are fortified with pyridine nucleotides (Davison 1955, Murphy & Dubois 1957, O'Brien 1957a). Degradation of such compounds by hydrolases should be carried out by slices or minces as well as by homogenates.

Two types of preparations were therefore used in the present study: (a) minces or slices: the effect of these preparations would be the overall result of both activating and degrading systems; (b) homogenates: these should show only degrading activity.

The results for mouse, rat, and ox liver slices are shown in figure 2. It is clear that the overall effect in the liver of the Co-ral resistant species, ox and rat, is to degrade, while that of the mouse, a susceptible species, is to activate. The effect of liver homogenates of these three species is shown in figure 3. The results demonstrate that

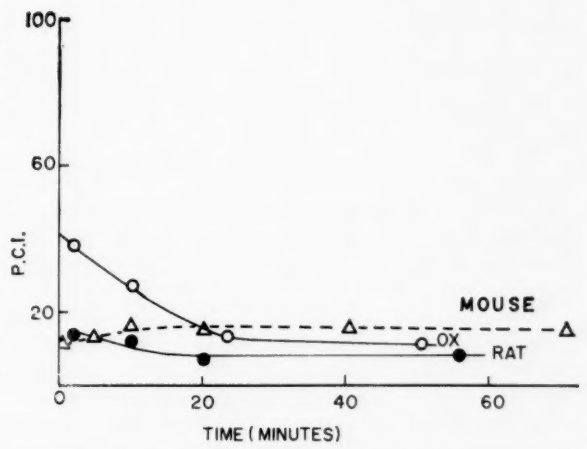

Fig. 3.—Effect of liver homogenates upon Co-ral solution. P.C.I. = per cent cholinesterase inhibition.

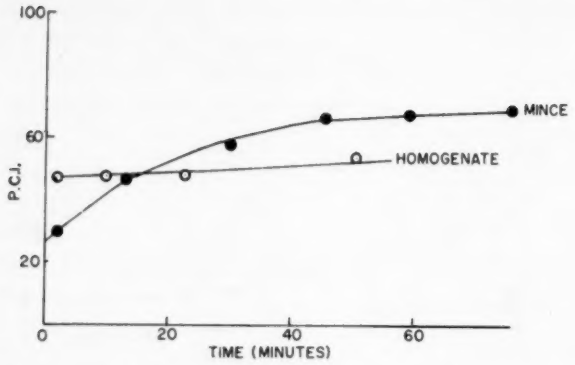

FIG. 4.—Effect of minces and homogenates of whole mouse upon Co-ral solution. P.C.I. = per cent cholinesterase inhibition.

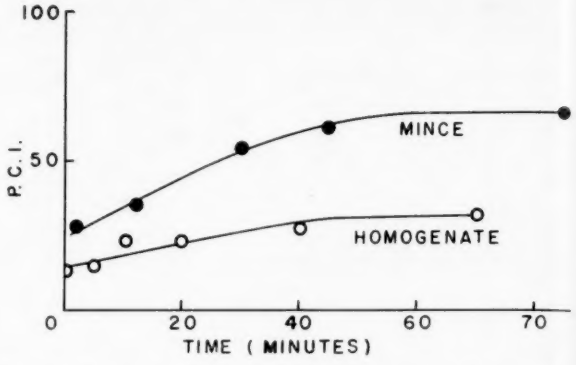

FIG. 5.—Effect of minces and homogenates of whole house fly upon Co-ral solution. P.C.I. = per cent cholinesterase inhibition.

there is degradative action by the liver of cow and (to a small extent) of rat, but not by that of mouse.

In case other tissues than the liver should be of importance, other preparations were examined. Whole minced mouse activated Co-ral, and even whole mouse homogenates had a small activating effect (fig. 4). It seems unlikely that any very active Co-ral degrading enzymes are present in the mouse. For the rat, a homogenate of kidney plus alimentary canal and associated organs was prepared, containing 3 gm. of tissue per 50 ml. of buffer; the preparation had no effect upon Co-ral. The following ox tissues (at 1.5 gm. of each homogenized in 25 ml. of buffer) had no effect on Co-ral: spinal cord, ventricle, kidney, serum.

So far it was clear that the nonsusceptible species could degrade Co-ral, and that in their liver slices there was a net degradation. Was this because no activating system was present, or was it the result of degradation outstripping activation? The sites of activation of phosphorothionates by liver are the microsomes (Davison 1955, O'Brien 1959). Ox liver microsomes were prepared by centrifuging at 45,000 xg the supernatant from a 10% homogenate of liver spun at 10,000 xg (all centrifugations at 2° C.). Microsomes derived from 200 mg. of liver were added to Warburg flasks with Co-ral (4×10^{-5} M) and Mg^{++}, nicotinamide, reduced diphosphopyridine nucleotide, and plasma cholinesterase as previously described (O'Brien 1957a), and the cholinesterase activity was assayed. The cholinesterase was 38% inhibited with Co-ral plus microsomes, compared to 12% without microsomes. Clearly an activating system is present in ox liver microsomes.

The effect of homogenates of rat, mouse, and ox livers upon coroxon was examined by adding 1 ml. of 5×10^{-4} M coroxon to homogenates and withdrawing samples for cholinesterase assay as in the Co-ral studies. No effect upon the coroxon was found over the 70-minute period studied.

Metabolism by Insect Tissue.—Minces of whole fly were potent in activating Co-ral (fig. 5). As in the mouse, even homogenates had a small activating effect, suggesting that degradative activity in this species is negligible.

In view of the findings up to this point that Co-ral-susceptible animals all activated Co-ral, it was expected that cattle grubs would show activation by this technique. The following larval tissues added to 25 ml. of buffer, failed to show any Co-ral activation: (a) 7 larval guts, chopped (700 mg.); (b) fat body from 7 larvae (1 gm.); (c) remainder of 7 larvae after removal of gut and fat-body, chopped (3.5 gm.).

A second technique was therefore employed. Larvae were injected with 50 mg./kg. of Co-ral suspended in 0.9% NaCl containing 0.5% Triton X-100, at the rate of 12.5 μl. per gm. of larva. Before injecting, the larvae were pierced to release some haemolymph, otherwise "bleeding" after injection was severe. The larvae reacted to the injection by contracting within 1 minute. They were then incubated at 35° in a water-saturated atmosphere. Pairs were taken at 10 minutes, 1 hour, 7 hours, and 24 hours, and each larva homogenized in 0.9% NaCl, 5 ml. per gm. live weight. All the larvae were alive before homogenizing, as judged by contractile activity. The homogenates were assayed against cholinesterase in the usual manner. There was negligible increase in anticholinesterase content (average per cent inhibition, for times given above: 4, 3, 6, 8). Attempts to inject 100 mg./kg. of Co-ral killed a number of the larvae, and again no increase in anticholinesterase content was found (only two times were studied: 20 minutes and 7 hours from injection).

A third technique was used. Larvae were opened by a single dorsal cut along the entire length, pinned in pairs in an insect perfusion bath (O'Brien 1957b) and perfused with 50 ml. of the standard buffer to which 1 ml. of Co-ral, 10^{-2} M in ethanol, was added. Samples were taken for assay as in the techniques described above. No activation was found in the 110 minute period studied.

The fourth technique was one that had proved successful in studying schradan activation (O'Brien & Spencer 1953). The insect tissue was added directly into Warburg flasks containing the buffered cholinesterase as used in all the assays above, and 0.5 ml. of 10^{-3} M Co-ral in 10% ethanol was added. After shaking gently for 1 hour on the platform of a wrist-action shaker, the flasks were gassed and the cholinesterase assayed as before, acetyl choline being tipped in precisely 30 minutes after the Co-ral addition. A clear activation was observed (table 1).

The possible presence of degrading systems was studied by homogenizing 1.5 gm. larvae in 25 ml. of buffer as in the mammalian studies. No degradation of Co-ral was observed.

DISCUSSION.—As far as the mammal is concerned, the

Table 1.—Activation of Co-ral by cattle grub tissues.

Tissue	Weight (mg.)	Per Cent		
		ChE. Inhibition[a] Alone = A	ChE. Inhibition with Co-ral[b] = B	Extra Inhibition Caused by Tissue Activation[c]
Nil		0	25	—
Fat Body	47	0	48	23
Gut	66	4	42	13
Remainder	205	1	33	7

[a] Mean of 2 values.
[b] Mean of 4 values.
[c] (B − A)—inhibition due to Co-ral.

liver is the only organ (of those studied) of importance in Co-ral metabolism. There appears to be an excellent correlation between liver-slice action and Co-ral toxicity: the susceptible mammal (mouse) activates, the resistant (ox, rat) degrade Co-ral. In the mouse there appears to be no degrading enzyme; in the cow both degrading and activating systems have been shown, in homogenates and microsomes respectively, and here the overall effect is clearly due to the balance between two competing reactions.

Both the fly and the cattle grub appear to have activating but no degrading activity, but the activating system of the larva is weak compared with the fly, and was detected only by the fourth technique used. This method involves the incubation of about 40 mg. tissue in 2.5 ml. of fluid, 2×10^{-2} M with respect to Co-ral. Thus although the tissue:fluid ratio in this fourth technique is low, the Co-ral concentration is 50 times greater, and presumably this factor accounts for the success of the technique.

The failure to show coroxon degradation in liver may be due to the same factor. As coroxon is a potent anticholinesterase, it was used at 2×10^{-5} M with the tissue preparation, *i.e.* at 20 times less a concentration than Co-ral. In these experiments the phosphorus compounds are used as substrates for tissue degrading enzymes, and presumably there is less activity at low substrate concentrations.

In the cattle grub, activating capacity exists in both gut and fat-body. It is possible that the activity found in the "residue" of the larva was due to incompletely removed fat-body.

The results serve to explain why the rat and ox are resistant to Co-ral poisoning, while the mouse, house fly, and cattle grub are susceptible. One can also understand why oral dosing of cattle is ineffective in cattle grub control: in this procedure the liver inactivates Co-ral before it reaches the larva. In dermal applications the Co-ral reaches the larva before the liver, and only by this procedure is the larva exposed to Co-ral. However, if it is true that dermal applications to the back will control oesophageal larvae, one would have to postulate that Co-ral can be transported from the back to the oesophagus without entering the blood, *e.g.* via the lymph, or by licking. The results suggest that the larva converts its own Co-ral, and is not killed by coroxon produced by the host.

These *in vitro* conclusions are therefore compatible with the facts *in vivo*. However, no attempts have been made to obtain optimal conditions, and it is possible that some of the reactions (particularly coroxon degradation) which we could not demonstrate might be of importance *in vivo*. It would be desirable to extend the observations in cattle grubs to first and second instars, since the third instar is not well controlled by Co-ral (MacDougall 1958).

References Cited

Aldridge, W. N. 1950. Some properties of specific cholinesterase with particular reference to the mechanisms of inhibition by E605 and analogues. Biochem. Jour. **46:** 451–60.

Chemagro Corporation. 1957. Bulletin announcing availability of Co-ral for experimental use. 4 pp.

Davison, A. N. 1955. The conversion of schradan (OMPA) and parathion into inhibition of cholinesterase by mammalian liver. Biochem. Jour. **61:** 203–9.

Krebs, H. A., and Henseleit, K. 1933. Untersuchungen über die Harnstoffbildung im Tierkorper. Zeitschr. physiol. Chem. **210,** 33–66.

Krueger, H. R., J. E. Casida, and R. P. Niedermeier. 1959. Bovine metabolism of organophosphorus insecticides IV. Metabolism and residues associated with dermal application of Co-ral (*O-O*,diethyl *O*-(3-chloro-4-methyl-7-coumarinyl) phosphorothioate) to rats, a goat and a cow. Jour. Agric. Food Chem. **7:** 182–8.

Lindquist, D. A., E. C. Burns, C. P. Pant, and P. A. Dahm. 1958. Fate of P^{32} labelled Bayer 21/199 in the white rat. Jour. Econ. Ent. **51**(2): 204–6.

MacDougall, D. 1958. Private communication.

March, R. B., T. R. Fukuto, R. L. Metcalf, and M. G. Maxon. 1956. Fate of P^{32} labelled malathion in the laying hen, white mouse and American cockroach. Jour. Econ. Ent. **49**(2): 185–95.

Murphy, S. D., and K. P. Dubois. 1957. Enzymatic conversion of the dimethoxy ester of benzotriazine dithiophosphoric acid to an anticholinesterase agent. Jour. Pharmacol. **119:** 572–83.

O'Brien, R. D. 1957a. Properties and metabolism in the cockroach and mouse of malathion and malaoxon. Jour. Econ. Ent. **50**(2): 159–64.

O'Brien, R. D. 1957b. Esterases in the semi-intact cockroach. Annals Ent. Soc. America **50:** 223–9.

O'Brien, R. D. 1959. Activation of phosphorothionates by liver microsomes. Nature **183:** 121–2.

O'Brien, R. D., and E. Y. Spencer. 1953. Metabolism of octamethylpyrophosphoramide by insects. Jour. Agric. Food Chem. **1:** 946–50.

O'Brien, R. D., G. D. Thorne, and R. W. Fisher. 1958. New selective insecticides developed on rational principles. Jour. Econ. Ent. **51**(5): 714–8.

Roth, A. R., and G. W. Eddy. 1955. Tests with some phosphorus compounds against cattle grubs. Jour. Econ. Ent. **48**(2): 201–2.

Smith, C. L., and R. Richards. 1954. New insecticides for control of the cattle grub. Jour. Econ. Ent. **47**(4): 712–3.

The Toxicity of Malathion to Killifish (Cyprinodontidae) in Delaware[1]

Richard F. Darsie, Jr., and F. Eugene Corriden[2]

Abstract

A series of field tests was performed to ascertain the extent of malathion toxicity to killifish (Family Cyprinodontidae) inhabiting tidal marshes in Delaware. The technique involved placing 25 fish in metal tubs containing 7 gallons of habitat water. Each treated tub had an untreated control. In all, 304 killifish of the species *Fundulus ocellaris* Jordan and Gilbert were tested. Sprays were delivered by aerial application to simulate practical mosquito control procedure. Malathion was applied at 0.5 pound per acre. The fish were exposed to the oil solution for 4 hours, after which time they were examined and classed into normal, moribund, and dead. Five tests were performed; in the last three, the moribund individuals were placed in a recovery tub of fresh habitat water and inspected 20 and 64 hours later. According to the 4-hour results, 26.3% died and 31.2% were unaffected, whereas 42.4% were sublethally poisoned. In four replicates the fate of the moribund fish was followed for 64 hours after treatment. Of these, 66% recovered while 26% died. Only 8% still were exhibiting symptoms at the end of the tests. There was no correlation between size of the fish and mortality.

A rather intensive study of the insecticide malathion for use in mosquito control in Delaware was begun in 1957, although a previous introductory evaluation was made in 1953 by MacCreary & Darsie (1954). A preliminary report of its 1957 performance was published by Darsie (1958). During the course of these and subsequent field tests on its toxicity both to mosquitoes and muskrats it was noted that some killifish (Family Cyprinodontidae) inhabiting the test plots were poisoned. Consequently, a series of controlled field tests was performed to ascertain more accurately the extent of this malathion toxicity.

Most of the previous work on the toxicity of malathion to fish has been in the form of laboratory tests with species other than killifish. Wood (1953) reported this toxicant at 5 p.p.m. lethal to fingerlings of rainbow trout, yellow perch, bluegills, and sunfish in less than 8 hours and to goldfish in less than 24 hours. The median lethal concentration to fall Chinook salmon fingerlings was determined to be about 0.1 p.p.m. by Parkhurst & Johnson (1955). Three p.p.m. of malathion killed 60% of carp in an experiment by Hayes (1955). The toxicity of organic phosphorus insecticides to the fathead minnow, *Pimephales promelas* Rafinesque, was explored by Henderson & Pickering (1957). They found that the median tolerance limit (LD-50) in p.p.m. was as follows: 24 hours, 25; 48 hours, 23; and 96 hours, 22. There was no difference in toxicity owing to hard or soft dilution water. Applegate *et al.* (1957) listed malathion as causing death to fingerling rainbow trout, bluegills, and sunfishes at five p.p.m. in less than 1 hour, but as having no effect on sea lamprey larvae.

There is very little accurate knowledge about the reaction of killifish to malathion. Cope & Springer (1958) reviewed this information as a part of a discussion on the effects of mass control of the Mediterranean fruit fly (*Ceratitis capitata* (Weid.)) on fish and wildlife. Field observations by personnel of the U. S. Department of Agriculture and Florida State Board of Health, following aerial applications of malathion from 0.2 to 0.75 pounds per acre, indicated extensive mortality to killifishes. Species involved were *Cyprinodon variegatus* Lacépède, *Fundulus confluentus* Goode and Bean, *Gambusia holbrooki* (Baird and Girard) and *Mollienesia latipinna* Le Sueur.

Procedure.—A series of five tests was completed during June, July, and August 1958. The marsh on which the fish were sprayed is located near Fennimore Bridge, 3½ miles east of Odessa, Delaware. In all, 304 ocellated killifish, *Fundulus ocellaris* Jordan and Gilbert were tested. They were caught in tidal streams with a 4- by 6-foot, ¼-inch mesh, common-sense seine. Three treated and three untreated replicates were used in each test. Replicates consisted of bushel-sized galvanized steel tubs containing 7 gallons of habitat water and approximately 25 fish selected at random. The experimental design consisted of a treated and control tub, replicated three times. There were three test sites, each containing one test and one control tub, and situated about 150 feet apart. Prior to use in these tests, the tubs were painted with DuPont dulux galvanized primer to prevent the fish from coming in contact with the metal and to reduce rusting. Between tests the tubs were scrubbed thoroughly with strong detergent to eliminate insecticide residues. They were filled with habitat water and fish approximately 12 hours before treatment, then buried one-half their depth in mud, covered with ¼-inch hardware cloth, and muslin for shade. At the time of spraying, the cloth and wire were removed from the tubs to be treated, while covers on the control tubs remained in place and in addition were protected by several thicknesses of wet newspapers.

One hour after the insecticide application, the wire and cloth covers were replaced on the sprayed tubs. Mortality in all tubs was recorded after 4 hours.

The toxicant was sprayed by a biplane, equipped with a lower wing boom calibrated to deliver 2 quarts of liquid per acre. Malathion was formulated by adding the 95% technical material to equal parts of fuel oil and an auxiliary solvent, Solvacide 544B (Socony Vacuum Oil Co.). The application rate in all tests was 0.5 pound per acre. Deposition of insecticide was recorded by placing carbon-coated glass slides near each test site.

After 4 hours' exposure to the insecticide the numbers of normal, moribund, and dead were recorded. Normal fish were defined as those which struggled to be free when captured in the hands, or those which could not be caught without the use of a minnow bucket. Moribund fish were

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defined as those which were alive and could be taken in the hands without attempting to get away. Fish in these three categories were separated and preserved in 10% formalin. In the laboratory total length of every specimen was measured. Only the 4-hour count was made in the first two tests.

For the last three tests, a third recovery tub was added at two of the three test sites. This tub was filled with 7 gallons of habitat water, at the same time as the others, prior to treatment. It was handled in the same manner as the control tub except that no fish were added at the start. The fish that were considered moribund after 4 hours were placed in this recovery tub. Then subsequent mortality counts were made 20 and 64 hours later.

The experimental data were analyzed by means of Student's *t* test in order to compare the mortality of treated and untreated fish after 4 hours' exposure to malathion. This test was used likewise to test differences in size of fish normal and dead at 4 hours.

RESULTS.—The insecticide coverage of the test area, as determined by the carbon-coated slides, resulted in an average number of droplets per square inch in all tests of 167.2. The mass median diameter of these droplets was 180.2 microns. Inadequate coverage of the third replicate in both the August 1 and 14 applications was indicated by the fact that the appropriate slides showed only 38 and 29% respectively, of the amount delivered to the other replicates.

The fate of the killifish after 4 hours' exposure to oil solutions of malathion is listed in table 1. The percentage killed outright in 12 replicates was 26.3. This result was compared by a *t* test with the controls, in which there was no mortality, and found to be significant at the 1% level. Of the remainder, 31.2% of the killifish were normal and 42.4% were showing sublethal poisoning.

Initial observations indicated that there was no correlation between the size of the fish and mortality. To substantiate this fact, the total length of the fish in each category was tabulated in table 2. The *t* test was calculated for the nine replicates in which the dead fish could

Table 1.—Toxicity of malathion to killifish after four hours' exposure. Delaware 1958.[a]

		Treated				Untreated	
			After 4 Hours' Exposure				
Date of Test	Replicate	Number Tested	Normal	Moribund	Dead	Number Tested	Normal After 4 Hours
June 18	1	25	17	1	7	24	24
	2[b]	(27)		(27)		(26)	(26)
	3	25	7	3	15	25	25
July 1	1	26	8	7	11	26	26
	2	25	10	14	1	25	25
	3	24	7	11	6	25	25
July 15	1	26	6	8	12	25	25
	2	25	1	12	12	25	25
	3	25	6	14	5	24	24
Aug. 1	1	27	20	7		25	25
	2	26	3	19	4	25	25
	3[c]	(25)	(24)	(1)		(26)	(26)
Aug. 14	1	25	6	17	2	24	24
	2	25	4	16	5	26	26
	3[c]	(25)	(25)			(25)	(25)
Total		304	95	129	80	298	298
Per cent of total			31.2	42.4	26.3		100
Average		25.3	7.9	10.7	6.6	24.8	24.8

[a] *t* test was calculated, comparing mortality in treated and untreated replicates: $t_{(.01)}$ with 11 d.f. = 3.106 < 4.394, therefore the difference is significant.
[b] No distinction made between moribund and dead fish; not included in totals and averages.
[c] Poor insecticide coverage was due to insufficient amounts in airplane to finish plot. The coverage which was obtained resulted from drift; not included in totals and averages.

Table 2.—Distribution of killifish by size in millimeters in relation to their susceptibility to malathion. Delaware 1958.[a]

		Treated					Untreated	
		Total		Average Length 4-Hour Exposure Data			Total (All Normal after 4 Hours)	
Date of Test	Replicate	Number	Average Length	Normal	Moribund	Dead	Number	Average Length
June 18	1	25	63.1	64.7	81.0	58.8	24	62.0
	2[b]	(27)	(56.4)		(56.4)		(26)	(57.3)
	3	25	56.6	55.4	52.6	58.0	25	66.8
July 1	1[c]	(26)	(51.3)	(53.2)	(50.4)		(26)	(53.3)
	2[c]	(25)	(59.3)	(62.3)	(57.3)		(25)	(55.5)
	3[c]	(24)	(59.1)	(59.5)	(58.9)		(25)	(57.1)
July 15	1	25	46.3	53.0	53.2	38.9	25	48.2
	2	25	49.0	55.0	51.8	45.7	25	50.4
	3	25	53.1	55.6	56.0	42.0	25	52.8
Aug. 1	1	27	40.1	40.8	38.2		25	39.0
	2	26	46.9	39.6	51.1	32.5	24	46.6
	3[d]	(24)	(47.2)	(47.2)	(61.0)		(25)	(44.8)
Aug. 14	1	24	47.1	45.3	48.5	41.5	24	41.3
	2	25	47.8	49.0	49.0	43.0	26	51.3
	3[d]	(25)	(43.5)	(43.5)			(25)	(46.6)
Average		25.2	49.9	51.3	50.6	47.3	24.7	50.9

[a] *t* test was calculated, comparing average length of normal and dead fish: *t* (.05) with 8 d.f. = 2.306 > 1.464, therefore the difference is not significant.
[b] Same as Footnote 2, Table 1.
[c] No separation of moribund and dead fish; not used in averages.
[d] Same as Footnote 3, Table 1.

Table 3.—Fate of moribund killifish 20 hours after exposure to malathion. Delaware 1958.

Date of Test	Replicate	Treated: Number Moribund	Treated: Average Length in mm.	Treated: 20 Hours After 4 Hours' Exposure: Normal	Moribund	Dead	Untreated: Number	Average Length in mm.	Normal After 24 Hours
July 15	1	8	53.2	2	2	4	—[a]		
	2	12	51.8	2	7	3	—[a]		
Aug. 1	1	7	38.2		6	1	25	39.0	25
	2	19	51.1		14	5	25	46.6	25
Aug. 14	1	17	48.5		14	3	24	41.3	24
	2[b]	(16)	(49.0)	?	?	0	(26)	(51.3)	(26)
Total		63		4	43	16	74		74
Per cent of total				6.3	68.2	25.3			100
Average		12.6	48.5	0.8	8.6	2.6	24.6	42.3	24.6

[a] Untreated checks were preserved after 4 hours.
[b] No distinction was made between normal and moribund fish, not included in totals and averages.

be compared with the normal ones. Significant differences owing to size were not demonstrated.

As the tests progressed, interest in the fate of the moribund fish increased. Did they recover from the poisoning, and if so, how much time was required? To explore these questions, therefore, recovery tubs were introduced at two of the sites after the second test. The normal and dead fish were placed in formalin at the time of the 4-hour count and only the moribund individuals were studied further. Results of the 20-hour examination are recorded in table 3. Most of the fish were still affected by the poison; however, in the six replicates 16% died. Again the normal and dead were withdrawn and only the moribund carried through to 64 hours. Of the 50 fish (in 4 replicates) still moribund at 20 hours, 66% recovered, 8% were still moribund, and 26% had succumbed (see table 4). The controls which were carried through the 68 hours, had but one dead and one moribund. A cumulative record of the malathion toxicity over the 68 hours is presented in table 5. Four replicates in two tests are included. About 10% of the total fish died initially and 57% were poisoned sublethally. It must be pointed out here that in the larger sample (12 replicates) reported in table 1, a higher percentage died after 4 hours and a proportionately smaller number were moribund. At 20 hours none of the moribund fish had recovered, whereas 10% had died. By 64 hours, 33 of the original 59 fish poisoned were normal, while only 22 had succumbed and 4 remained in moribund condition. Considering the 64% of the total test fish, which were normal after 64 hours, then, 32% were unaffected originally and 32% had shown poisoning symptoms and recovered.

DISCUSSION.—The tests point out that malathion is toxic to the ocellated killifish. The extent of mortality did not exceed 33%, while another 33% were not affected at all by the dosage employed. The fate of those individuals that were poisoned, but remained alive, is in question. The tests indicated that 56% of these would recover if transferred to fresh water following the exposure period. It must be emphasized that no additional changes of fresh water were made during the 64-hour recovery period. Undoubtedly some malathion was transferred with the fish from the treated tubs to recovery tubs. Therefore, the number of fish revived probably would be greater under more ideal conditions.

The symptoms of malathion poisoning were evident in both the free-living and captured killifish. The first sign to be noted was their lethargy. They could be touched without any attempt to swim away. In more severe poisoning the body, and particularly the caudal peduncle,

Table 4.—Fate of moribund killifish 64 hours after exposure to malathion. Delaware 1958.

Date of Test	Replicate	Treated: Number Moribund	Treated: Average Length in mm.	Treated: 64 Hours After 4 Hours' Exposure: Normal	Moribund	Dead	Untreated: Number	Average Length in mm.	After 68 Hours: Normal	Moribund	Dead
Aug. 1	1	6	38.9	6			25	39.0	25		
	2	14	52.7		3	11	25	46.6	25		
Aug. 14	1	14	48.9	13		1	24	41.3	24		
	2	16[a]	49.0	14	1	1	26	51.3	24	1	1
Total		50		33	4	13	100		98	1	1
Per cent of total				66.0	8.0	26.0			98.0	1.0	1.0
Average		12.5	47.3	8.5	1.0	3.2	25	44.5	24.5	0.2	0.2

[a] Some may have been normal at 20 hours.

Table 5.—Cumulative record of the toxicity of malathion to killifish. Delaware 1958.

Date of Test	Replicate	Number Tested	Total: After 4 Hours' Exposure: Normal	Total: After 4 Hours' Exposure: Moribund	Total: After 4 Hours' Exposure: Dead	Cumulative Total: 20 Hours After Exposure: Normal	Cumulative Total: 20 Hours After Exposure: Moribund	Cumulative Total: 20 Hours After Exposure: Dead	Cumulative Total: 64 Hours After Exposure: Normal	Cumulative Total: 64 Hours After Exposure: Moribund	Cumulative Total: 64 Hours After Exposure: Dead
Aug. 1	1	27	20	7	0	20	6	1	26	0	1
	2	26	3	19	4	3	14	9	3	3	20
Aug. 14	1	25	6	17	2	6	14	5	19	0	6
	2	25	4	16	5	4	16[a]	5	18	1	6
Total		103	33	59	11	33	50	20	66	4	33
Per cent of total			32.0	57.2	10.6	32.0	48.5	19.4	64.0	3.8	32.0
Average		25.7	8.2	14.7	2.7	8.2	12.5	5.0	16.5	1.7	8.2

[a] No distinction made between moribund and normal fish at 20 hours.

was bent laterally and held in that position. Figure 1 shows the appearance of normal and poisoned ocellated killifish.

What actually happens to the free-living, poisoned fish remains unknown. It was noticed that, by the next high tide, there was no sign of moribund or dead fish. What effects this depletion had on the total population, and how rapidly its restoration to previous levels occurred, is unanswered. This area was visited both in September and October following the last spray, at which time there appeared to be no numerical difference in killifish within the treated area and on other parts of the marsh.

FIG. 1.—(A) Normal and (B) malathion-poisoned ocellated killifish, *Fundulus ocellaris*. The largest specimen was 76 mm. long.

Another consideration is the possible secondary effect due to predation on morbid fish. Shore birds particularly would be attracted to the easily captured prey. Some indications of the susceptibility of birds to malathion are contained in the work of Furman & Weinmann (1956). They found that 9-month-old chickens and 12- to 15-week-old turkeys could withstand dips of malathion up to 1%. Much experimentation on control of ectoparasites has demonstrated the relatively nontoxic nature of this chemical to poultry; *e.g.* Moore & Schwardt (1954), Harding (1955), Furman *et al.*, (1955), Raun (1956), Rodriguez & Riehl (1958) and Gless & Raun (1958).

Further research is needed to clarify the impact of malathion toxicity to killifish on its own population level and on the other animals ecologically related.

REFERENCES CITED

Applegate, Vernon C., John H. Howell, A. E. Hall, Jr., and Manning A. Smith. 1957. Toxicity of 4346 chemicals to larval lampreys and fishes. U. S. Fish and Wildlife Serv. Spec. Sci. Rept. No. **207,** 157 pp.

Cope, Oliver B., and Paul F. Springer. 1958. Mass control of insects: The effects on fish and wildlife. Bull. Ent. Soc. America **4**(2): 52–6.

Darsie, Richard F., Jr. 1958. The use of malathion in mosquito control, including its performance in Delaware during 1957. Proc. 45th Meeting New Jersey Mosquito Exterm. Assoc. pp. 101–111.

Furman, Deane P., Lloyd E. Vincent, and W. Stanley Coates. 1955. External parasites of poultry. California Agric. **9**(1): 13.

Furman, Deane P., and C. J. Weinmann. 1956. Toxicity of malathion to poultry and their ectoparasites. Jour. Econ. Ent. **49**(4): 447–50.

Gless, Elmer E., and Earle S. Raun. 1958. Insecticidal control of the chicken body louse on range turkeys. Jour. Econ. Ent. **51**(2): 229–32.

Harding, Wallace E., Jr. 1955. Malathion to control the northern fowl mite. Jour. Econ. Ent. **48**(5): 605–6.

Hayes, M. L. 1955. Evaluation of ryania and malathion as fish toxins. Colorado Coop. Fish Res. Unit, Quart. Rept. 1(3–4): 40–2.

Henderson, Croswell, and Quentin H. Pickering. 1957. The toxicity of organic phosphorus insecticides to fish. Paper of 80th Ann. Meet. Amer. Fish. Soc., pp. 1–32.

MacCreary, Donald, and Richard F. Darsie, Jr. 1954. Loss of DDT-resistance in Delaware's salt-marsh mosquito larvae, and results with BHC, EPN, and malathion in 1953. Proc. 41st Meet. New Jersey Mosquito Exterm. Assoc., pp. 218–20.

Moore, Steve, III, and H. H. Schwardt. 1954. The control of external parasites of chickens in New York State. Poultry Sci. 33(6): 1230–7.

Parkhurst, Z. E., and H. E. Johnson. 1955. Toxicity of malathion 500 to fall Chinook salmon fingerlings. Progr. Fish Culturist 17(3): 113–6.

Raun, Earle S. 1956. Chicken louse and mite control with malathion formulations. Jour. Econ. Ent. 49(5): 628–9.

Rodriguez, J. L., Jr., and L. A. Riehl. 1958. Malathion for control of chicken mites on hens in wire cages. Jour. Econ. Ent. 51(2): 158–60.

Wood, E. M. 1953. The toxicity of 3400 chemicals to fish. U. S. Fish and Wildlife Serv. Branch Fish. Biol. Unpubl. Rept., 196 pp.

Laboratory Tests for Control of the Beet Leafhopper on Snap Beans Grown for Seed[1]

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ABSTRACT

Many chemicals were tested, both as seed and foliar treatments, for control of the beet leafhopper (*Circulifer tenellus* (Bak.)) and consequent reduction in curly top on snap beans grown for seed. Outstanding results were obtained with a foliar spray containing 0.125% Thimet® (*O,O*-diethyl *S*-(ethylthio)-methyl phosphorodithioate) and 4% juice pressed from sugar beets highly resistant to curly top. Good control was also obtained with DDVP, lindane-DDT-Aroclor, DDT, dimethoate, and Thimet separately. The seed treatments were less satisfactory.

About 80% of the bush-type snap bean seed grown in the United States is produced in southern Idaho. This crop, valued at $3,000,000 to $4,000,000 annually, is very susceptible to curley top, a virus disease transmitted by the beet leafhopper (*Circulifer tenellus* (Bak.)). Some sections of southern Idaho, and often the entire area are affected by this disease every year.

A project to find a direct method of controlling the beet leafhopper on snap beans was started at Twin Falls, Idaho, in 1946, and the early work was reported by Hallock & Deen (1956). This paper covers the work on the project since 1953.

MATERIALS AND METHODS.—Bountiful beans, a variety very susceptible to curly top, were used in all the tests. Antibiotics, hormones, and systemic insecticides were tested as both seed treatments and foliage sprays. Nonsystemic insecticides were also tested in sprays at concentrations from 0.125 to 1% and in some of the preliminary tests in 10% dusts.

In the seed treatments, the chemicals were used as both slurry treatments and seed soaks. In a slurry treatment, a slurry of the test chemical, thiram, and methyl cellulose was mixed with the seed. Two ounces of thiram were used per 100 pounds of seed and a sufficient amount of 1.5% methyl cellulose solution to give an even seed coat. The seed soaking was accomplished by placing the seed in an aqueous preparation of thiram and the test chemical.

All the seed was sprouted in vermiculite and healthy plants were transplanted singly to 4-inch flower pots to obtain plants of a uniform size and age for testing.

Where either seed soaks or slurry treatments were used, the plants were inoculated the day after transplanting by caging four viruliferous beet leafhoppers that had been without food for at least 4 hours on each plant. The cage used permitted the insects to feed on the entire plant. After 2 days the leafhoppers and cages were removed.

Foliar treatments were made 1 day after transplanting to individual plants as they revolved on a turntable (fig. 1) described by Peay & Oliver (1957). In preliminary tests dusts were applied at the rate of 0.5 gram per plant as it revolved under a bell jar. Sprays were applied without the bell jar at 5 cc. per plant with a DeVilbiss spray gun (type CV) at 10 pounds' pressure, the plants being wet to the dripping stage. Ten plants treated with each material constituted a test, and if enough material was available, at least 4 tests were made. After the treatment the plants were set aside for 1 day and then inoculated as described above. At the end of 2 days leafhopper mortality counts were made. The cages were then removed and weekly curly-top readings made for at least 30 days. These disease readings were used in the final evaluation of the materials, because beet leafhoppers do not live long even on untreated bean plants. Furthermore, unless a material protects the plants from curly top, it is of no value on beams.

RESULTS.—The antibiotics Bacitracin, Polymixin, streptomycin, and Terramycin were used as slurry seed treatments at rates from 1 to 16 ounces per 100 pounds of seed and as seed soaks for 1 to 8 hours in a solution of 800 parts per million. No control of either the beet leafhopper or curly top was obtained with these materials. Neither was control obtained when they were used as foliage sprays at 100 to 800 parts per million.

The hormone 2,4-dichlorophenoxyacetic acid (2,4-D) was used in concentrations from 5 to 640 parts per million as seed soaks for 15 to 120 minutes. It was also used as a

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[2] W. N. Oliver grew all the plants for these tests. W. A. McIntyre inoculated and removed the leafhoppers from the plants in some of the tests.

Table 1.—**Beet leafhopper mortality and curly top on Bountiful bean plants grown from seed soaked 2 to 4 hours in a 1% solution of insecticide.**

INSECTICIDE	PER CENT MORTALITY OF LEAFHOPPERS AFTER 2 DAYS	PER CENT OF PLANTS WITH CURLY TOP
Thimet	96.7	62.1
Dimethoate	96.6	70.1
Demeton	78.9	71.2
Schradan	48.5	87.6
Untreated check	46.5	89.7
L.S.D. at 5% level	14.16	9.1

spray at 5 to 40 parts per million. None of these treatments gave any reduction of leafhoppers or curly top, and the plants were distorted or killed by the heavier dosages. Gibberellic acid used as seed treatments and sprays at several concentrations gave no control of the leafhopper or curly top. In fact, it even hastened the development of the disease symptoms.

Four systemic insecticides were used as slurry seed coats at 4 to 32 ounces per 100 pounds of seed and as seed soaks for 1 to 8 hours in a 1% concentration in water. Table 1 shows that Thimet®, (*O,O*-diethyl *S*-(ethylthio) methyl phosphorodithioate), dimethoate, and demeton gave good kills of the beet leafhopper but very little reduction of curly top. Schradan was of no value. Thimet, the best material, gave 30% reduction of curly top but was toxic to the seed when used at more than 4 ounces per 100 pounds as a slurry treatment or when the seed was soaked longer than 4 hours in a 1% concentration.

The following materials gave no control of the leafhopper when applied as foliage sprays as judged by curly-top readings:

Aldrin
Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite)
Chlorobenzilate® (ethyl 4,4′-dichlorobenzilate)
Dieldrin
Dimethoxyphenyl trichloroethane
2,3-Dimethyl-1,4-dioxaspire [4,5] decane
Heptachlor
Delnav® (2-3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate)
Isodrin
2-Isopropyl-3,3-dimethyl-1,5-dioxaspire [5,5]undecane
Lindane
DL-Lysine monohydrochloride
3-Phenyl-*DL*-alamine
Pyrolan® (dimethyl 5-(3-methyl-1-phenyl-pyrazolyl) carbamate)
Sugar-beet juice
Sulfur
Sulphenone® (*p*-chlorophenyl phenyl sulfone)
Tetra-*n*-propyl dithionopyrophosphate
Toxaphene

The results with materials that gave partial control of either the leafhopper or curly top, or both, are given in table 2 and those that were the most outstanding are given in table 3.

Thimet's effectiveness in controlling the beet leafhopper, including its systemic action, indicated that it might be combined with some material that would control the disease. Of several such materials tested in the greenhouse, one showed outstanding effectiveness when combined with Thimet in a foliage spray. This material was juice pressed from sugar beets highly resistant to curly top.[3] However, when the combination was used as a seed treatment, it gave good reduction of beet leafhoppers but not of curly top. Sugar-beet juice has been tested alone several times since 1954 at various strengths from 0.25 to 40%, and it showed no promise for the control of either the leafhopper or curly top.

[3] The resistant sugar beets were obtained from A. M. Murphy, of the Crop Research Division, Agricultural Research Service, U.S.D.A.

Table 2.—**Insecticide sprays that gave partial control of either the beet leafhopper or curly top.**

INSECTICIDE	PER CENT CONCENTRATION	PER CENT REDUCTION	
		Leafhoppers	Curly Top
Alloxan	1.0	100	0
L-Arginine, monohydrochloride	0.25 –0.5	100	0
Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate)	1.0	100	67
Demeton	1.0	100	45
Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate	0.5 –1.0	100	61
O,O-Diisopropyl S-trichloromethyl phosphorothioate	.125–1.0	48	24
Dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate, acetate	.125–1.0	100	33
Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate)	.5	100	20
Ethion	.125–1.0	100	30
2-Ethyl-2-nitro-1,3-propanediol	1.0	100	13
4′-Formylacetanilide thiosemicarbazone	0.25 –0.5	95	22
L-(+)-Glutamic acid	.25 – .5	100	11
Guthion® (*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl)phosphorodithioate	.5	100	60
Hexaethyl(ethylthiomethylidyne)-triphosphonate	.25 – .5	100	37
L-(+)-Histidine, monohydrochloride	.25 – .5	100	33
p-Hydroxybenzaldehyde thiosemicarbazone	.25 – .5	81	17
Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol)	.125–1.0	75	3
dl-Methionine	.125–1.0	94	30
Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical)	.5	100	60
Phosphamidon	0.125–1.0	100	44
Rotenone 0.4% plus castor oil 4% in ethanol	1.0	100	21
dl-Serine	0.25 –0.5	100	11
Sevin® (1-naphthyl-N-methyl carbamate)	.5	100	—[a]
Sodium 8-hydroxy-(hydroxymercuri)-5-quinolinesulfonate	.25 – .5	100	25
Tetrachlorophthalic acid	1.0	67	7
Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide)	0.125–1.0	100	60
Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate)	.125–1.0	100	0

[a] This material burned the plants at the concentration used.

FIG. 1.—Apparatus used for applying insecticides to individual plants in tests for the control of the beet leafhopper on beans.

FIG. 2.—Left, Bountiful bean plants sprayed with sugar-beet juice combined with Thimet; right, untreated diseased plants. Both groups of plants were infested with curly-top infective leafhoppers.

Table 3.—**Percentage of beet leafhopper mortality and of bean plants developing curly top after treatment of foliage with various insecticides.**

Insecticide	Per Cent Concentration	Per Cent		
		Leafhopper Mortality	Of Plants with Curly Top	Reduction
Sugar-beet juice+Thimet	4.0+0.125	100	9	90.4
DDVP	1.0	100	14	85.1
Lindane-DDT-Aroclor	0.6	100	16	83.0
DDT	1.25	93	20	78.7
Dimethoate	.125	100	54	42.6
Thimet	.125	100	57	39.4
Sugar-beet juice	4.0	71	98	—
Untreated check	—	63	94	—
L.S.D. at 5% level	—	10.9	16.0	—

Figure 2 shows three bean plants sprayed with a mixture containing 0.125% of Thimet plus 4% of resistant beet juice, compared with untreated plants. This mixture has been consistently effective in the greenhouse where the beans were subjected to a single 2-day exposure to infective leafhoppers. In limited greenhouse tests on tomatoes, it has given excellent control of the leafhopper and a 91% reduction of curly top. It is not known how it will hold up under field conditions where the leafhoppers may continue to move into the field for many days.

Tests are currently being conducted to determine the effectiveness of the Thimet-sugar-beet juice combination in controlling the beet leafhopper and preventing the development of curly top when test plants are subjected to several inoculations by viruliferous leafhoppers under greenhouse conditions and to determine how much the sugar-beet juice concentrations can be reduced without affecting the results. As yet there has been no significant difference in the amount of curly-top reduction between the 5 and 40% concentrations.

References Cited

Hallock, H. C., and O. T. Deen. 1956. Greenhouse tests on control of the beet leafhopper. Jour. Econ. Ent. 49(1): 123–6.

Peay, Walter E., and William N. Oliver. 1957. An apparatus for applying dusts and sprays to small plants in the laboratory. U. S. Dept. Agric., **ARS-33-38,** 2 pp.

Comparative Residual Behavior of Pyrethrins and Piperonyl Butoxide on Wheat[1]

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Abstract

Because of difficulty in determining residues of pyrethrins quantitatively in the presence of high concentrations of the usual synergist, piperonyl butoxide, the evaluation of the residual behavior of the readily analyzed piperonyl butoxide alone has heretofore been thought to be adequate for most residue studies with pyrethrins-piperonyl butoxide mixtures. The residual behavior of the pyrethrins has been thought to parallel that of the piperonyl butoxide component of such mixtures.

By the use of chromatographic techniques in conjunction with colorimetric procedures, the residual behavior of the pyrethrins and of piperonyl butoxide on stored wheat have been compared. The pyrethrins have an apparent residue half-life of 5.8 weeks in contrast with that of 9.9 weeks for piperonyl butoxide.

Despite impressive advances in the field of pesticide residue methodology during the past 10 to 15 years, development of adequate methods for determining pyrethrins residues on foodstuffs has lagged. Cueto & Dale (1953) developed a colorimetric procedure utilizing the red-brown color developed when an alcoholic solution of pyrethrins was heated at 70° C. with a solution of sodium sulfide. Levy & Estrada (1954) developed a similar procedure with solutions of sulfur in alcoholic potassium hydroxide and in carbon tetrachloride for the color-developing reagents. Schreiber & McClellan (1954) proposed a colorimetric procedure for pyrethrin I, using the color changes produced by the mercury reduction method. Levy & Molina (1957) determined colorimetrically the methyl alcohol resulting from the hydrolysis of pyrethrin II. Williams *et al.* (1956) found that an intense rose-red color developed when pyrethrins were heated with ethyl acetate and phosphoric acid for 3 minutes.

The sulfide method is insufficiently sensitive and does not respond when piperonyl butoxide is present. The mercury reduction and methyl alcohol methods suffer from over-specificity for use with the usual multicomponent pyrethrins. The ethyl acetate-phosphoric acid method is sensitive and responds to both pyrethrin I and pyrethrin II, but only in the absence of piperonyl butoxide. Because of these difficulties, residue studies are often conducted on the piperonyl butoxide synergist, which is nearly always present in greater concentration than the pyrethrins and is therefore more easily analyzed, on the assumption that the residual behavior of the insecticide and of the synergist are parallel. It is the purpose of this paper to demonstrate differences in residual behavior of pyrethrins and of piperonyl butoxide on wheat.

Material and Methods. *Treatment of Grain.*—White Federation wheat of 13% moisture content was sprayed with 51.075 grams of a wettable-powder formulation containing 2% pyrethrins and 20% technical piperonyl butoxide per 3,900 ml. of water at the rate of 260 ml. of spray per 100 pounds of grain. The treated grain was stored in tightly sealed 100-pound lard cans at 90° F.

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The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

[2] The authors wish to thank R. G. Strong for supplying and treating the wheat and H. W. Seiss, Jr., for a portion of the analytical data.

Probe samples were collected at five points in each can 1, 14, 30, and 90 days after treatment. The resulting 500-gram sample was equilibrated in solvent for 30 minutes in a tumbler-type stripping apparatus (Gunther & Blinn 1955) with 1 ml. of *n*-hexane per gram of wheat. The resulting stripping solution was filtered and stored under refrigeration until analyzed.

Analytical Procedures.—The analytical procedure used on pyrethrins was essentially that described by Williams *et al.* (1956), except that chromatographic isolation was achieved before color development. For this purpose, a silicic acid–Hyflo Super-Cel (2:1) mixture, previously activated at 180° C. for 12 hours (Sweeney & Williams 1957), was used in a 20- ×100-mm. column with 10% ethyl ether in *n*-hexane as solvent. The first 40 ml. of eluate was discarded, and the next 50 ml. was collected for colorimetric analysis. To determine which pyrethrins were being eluted by this procedure, representative second fractions were chromatographed on paper, with *n*-hexane saturated with methyl alcohol as the solvent system. Spots at R_f 0.93 and 0.80 corresponding to pyrethrin I and pyrethrin II were found, using dilute permanganate solution for detection. No spots were found in pre- or post-eluates.

The analytical procedure for piperonyl butoxide was that described by Williams & Sweeney (1956). Chromatographic cleanup was achieved with a column (12×50 mm.) containing aluminum oxide Woelm, neutral grade I plus 2% water, with 40% methylene chloride in *n*-hexane solution to elute the piperonyl butoxide.

DISCUSSION.—From the data presented in table 1 residual half-life values (Gunther & Blinn 1955) of 5.8 weeks for pyrethrins and 9.9 weeks for piperonyl butoxide may be calculated in the usual manner. Piperonyl butoxide is much more persistent than pyrethrins under these conditions. Therefore, the practice of analyzing for piperonyl butoxide residues on the assumption that pyrethrins content maintains its original ratio with piperonyl butoxide is not valid. However, this practice can be condoned for purposes of defining toxicological hazard if the actual residual relationship of these two materials is considered.

Table 1.—Residues of pyrethrins and of piperonyl butoxide found on wheat of 13% moisture content, stored at 30° C.

Days After Treatment	Parts per Million	
	Pyrethrins[a]	Piperonyl Butoxide[b]
1	0.8	7.7
14	.6	6.2
30	.4	5.0
90	.2	3.5

[a] Average of two values. Corrected for background (0.1 p.p.m.) and for recovery (89%).
[b] Average of two values. Corrected for background (0.1 p.p.m.) and for recovery (98%).

REFERENCES CITED

Cueto, C., and W. E. Dale. 1953. Colorimetric determination of pyrethrins, allethrin, and furethrin. Analyt. Chem. **25:** 1367.

Gunther, F. A., and R. C. Blinn. 1955. Analysis of Insecticides and Acaricides. Interscience Publishers, New York-London.

Levy, L. W., and R. E. Estrada. 1954. Rapid colorimetric determination of total pyrethrins by reaction with sulfur. Jour. Agric. Food Chem. **2:** 629.

Levy, L. W., and H. Molina. 1957. Hydrolytic method for the estimation of pyrethrin II in pyrethrum flowers. Pyrethrum Post **4,**(2): 22.

Schreiber, A. A., and D. B. McClellan. 1954. Estimation of micro quantities of pyrethroids. Analyt. Chem. **26:** 604.

Sweeney, J. P., and H. L. Williams. 1957. Colorimetric method for the determination of cyclethrin. Jour. Agric. Food Chem. **5:** 670.

Williams, H. L., W. E. Dale, and J. P. Sweeney. 1956. A new colorimetric method for pyrethrins. Jour. Assoc. Offic. Agric. Chemists **39:** 872.

Williams, H. L., and J. P. Sweeney. 1956. Isolation of piperonyl butoxide from oils, fats, and waxes. Jour. Assoc. Offic. Agric. Chemists **39:** 975.

Evaluation of Some Space Sprays for the Control of House Flies[1]

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ABSTRACT

Laboratory tests were run to select promising insecticides and to determine practical concentrations for tests with space sprays against house flies (*Musca domestica* L.) in dairy barns. Tests in barns with three organophosphorus compounds, pyrethrins, and four chrysanthemumates usually gave at least 90% control in 10 minutes but little or no control at 24 hours. Daily spraying for 3 weeks caused only minor reductions in the abundance of flies.

In the last several years house flies (*Musca domestica* L.) have developed resistance to the common insecticides. In dairy barns residual treatments that formerly gave long periods of control when applied to the walls and ceilings are now completely ineffective. Other conventional methods, including the use of chlorinated hydrocarbon insecticides as space sprays, organophosphorus compounds as baits, and compounds of either type as larvicides, frequently fail to give any practical control.

In a search for more effective control measures, tests were made to evaluate several organophosphorus insecticides and chrysanthemumic acid derivatives as space sprays. A spray containing pyrethrum and piperonyl butoxide was used as a standard of comparison.

MATERIALS AND METHODS.—The insecticides were first tested in the laboratory. From four to six concentrations were tested in a wind tunnel, and then two or three concentrations in a spray chamber. On the basis of these results a single concentration was selected for tests in dairy barns.

The wind tunnel was a cylindrical tube 4 inches in diameter through which a column of air was moved at 4 m.p.h. by a suction fan. Twenty female flies 4 or 5 days

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Table 1.—Knockdown and mortality of house flies exposed to sprays in a wind tunnel and a spray chamber.[a]

Insecticide and Concentration (%)	Wind Tunnel				Spray Chamber		
	Per Cent				Per Cent		
	Knockdown at Indicated Minutes			Kill in 24 Hours	Kill in 24 Hours		
	10	30	60		Knockdown in 30 Min.	Knocked Down Flies	Total Flies
Diazinon[b]							
0.05	0	13	23	30	—	—	—
.1	5	45	55	63	96	100	96
.25	—	—	—	—	94	100	94
.5	—	—	—	—	100	100	100
1.0	33	100	100	100	—	—	—
Dipterex[c]							
0.1	0	0	3	15	—	—	—
.5	0	0	0	38	—	—	—
1.0	0	0	10	98	15	100	15
2.0	0	13	78	95	—	—	—
Ronnel							
0.1	0	0	0	40	—	—	—
.5	0	0	15	95	—	—	—
1.0	0	0	38	100	62	100	62
2.0	—	—	—	—	84	100	84
Malathion							
0.1	0	0	8	15	—	—	—
.5	0	5	18	50	—	—	—
1.0	0	0	20	63	74	97	72
2.0	3	58	95	95	84	100	84
Allethrin[d]							
0.0125	3	5	0	3	—	—	—
.025	8	5	3	0	—	—	—
.05	51	52	37	4	—	—	—
.1	87	95	94	52	51	73	37
.2	—	—	—	—	98	100	98
.25	100	100	100	100	—	—	—
Barthrin (6-chloropiperonyl chrysanthemumate							
0.1	0	0	0	3	—	—	—
.25	13	13	13	13	—	—	—
.5	20	33	25	20	—	—	—
1.0	83	95	98	90	89	100	89
2.0	100	100	100	100	94	100	94
6-Bromopiperonyl chrysanthemumate							
0.1	3	3	0	0	—	—	—
.25	5	8	3	8	—	—	—
.5	15	48	40	5	—	—	—
1.0	63	90	88	73	89	100	89
2.0	78	98	98	93	94	100	94
m-Isopropoxybenzyl chrysanthemumate							
0.1	0	0	0	3	—	—	—
.25	0	0	0	8	—	—	—
.5	0	0	0	3	—	—	—
1.0	18	20	25	10	77	99	76
2.0	40	75	40	25	90	98	88
m-(2-Methylallyloxybenzyl) chrysanthemumate							
0.1	3	3	3	3	—	—	—
.25	0	0	0	8	—	—	—
.5	0	0	0	0	—	—	—
1.0	0	0	3	5	51	90	45
2.0	15	63	50	8	38	79	30
Pyrethrins[d]							
0.01	100	98	90	33	—	—	—
.025	100	100	98	53	—	—	—
.05	100	100	100	83	—	—	—
.1	100	100	100	100	100	100	100
.2	—	—	—	—	94	97	81

[a] Based on two tests.
[b] *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.
[c] *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate.
[d] Synergized with 10 times as much piperonyl butoxide.

old were confined in a cylindrical screen cage and placed in the center of the tube. One-fourth milliliter of a solution of the insecticide in odorless kerosene was atomized at a pressure of 1 p.s.i. into the mouth of the tunnel, and the flies were exposed momentarily as the spray was drawn through the cage. After treatment the flies were transferred to untreated screen-holding cages and furnished honey solution. The mortality was recorded after 24 hours.

The spray chamber, containing approximately 150 cubic feet, was fitted with an exhaust fan to remove the sprays from the air at the conclusion of each test. The walls, ceiling, and floor were completely covered with brown wrapping paper, which was changed after each test while the room was being ventilated. In each test 50 female flies were released in the room and given 30 minutes to become adjusted. An odorless kerosene solution of the insecticide was then sprayed into the chamber as a fine mist at the rate of 1 ml. for each 50 cubic feet. Thirty minutes later the knocked-down flies were counted, placed in a clean holding cage, and held for 24-hour mortality counts.

RESULTS.—The results of these tests are given in table 1. With six toxicants the total mortalities in the spray-chamber tests were very close to those obtained with the same concentrations in the wind tunnel, but they were lower with two organophosphorus compounds and higher with two chrysanthemumates. Some of the flies knocked down with pyrethrins, allethrin, and the other chrysanthemumates recovered in 24 hours, particularly in the wind tunnel.

Seven insecticides selected from the laboratory tests and an additional chrysanthemumate were tested against natural populations of house flies in dairy barns. Solutions in odorless kerosene were applied 5 days a week at 1 ml. per 50 cubic feet with an electric mist sprayer. All fly-infested areas within each barn were treated. Estimates of fly abundance were made by counting the flies feeding on cloth strips (1 by 24 inches) saturated with a 50% malt solution. Counts were made before and at various intervals after treatment.

The results for the first week are given in table 2. All the treatments caused considerable knockdown in 10 minutes, usually more than 90%, but none gave good control after 24 hours. Populations remained high even when the program was extended to 3 weeks.

Table 2.—Control of house flies with space sprays applied in dairy barns on 5 successive days.

Insecticide and Concentration (%)	Pre-treatment Count	Per Cent Reduction: In 10 Minutes on Indicated Day 1	2	3	4	5	In 24 Hours on Indicated Day 1	2	3	4
Diazinon 0.1	92	82	90	91	91	95	11	0	0	10
	82	94	95	85	81	87	0	0	0	0
Ronnel 2.0	250	71	70	79	90	—	0	2	0	0
	40	—	—	—	—	—	55	38	33	65
Malathion 2.0	212	85	92	97	97	—	0	23	29	—
	20	—	—	—	—	—	0	0	5	—
Allethrin[a] 0.1	74	100	76	98	99	—	72	8	6	0
	213	—	98	98	93	99	0	0	0	—
Barthrin 2.0	43	95	97	96	—	—	0	0	0	—
	126	91	97	98	98	98	0	0	0	—
6-Bromopiperonyl chrysanthemumate 2.0	78	78	93	96	98	98	0	4	12	33
	86	97	91	91	95	93	0	21	25	0
2,4-Dimethylbenzyl chrysanthemumate 2.0	199	98	99	99	99	97	0	0	0	0
	200	98	96	97	96	96	0	0	0	0
Pyrethrins[a] 0.1	227	98	99	97	98	98	52	38	0	21
	151	—	—	—	—	—	0	0	0	0

[a] Synergized with 10 times as much piperonyl butoxide.

Effect of Methyl Bromide and Hydrocyanic Acid Fumigation on the Germination of Rice[1]

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ABSTRACT

Two varieties of rice, Calrose and Caloro, with a graded moisture range of 8, 10, 12, and 14%, were fumigated with methyl bromide or hydrocyanic acid in 100-cubic-foot gastight chambers. Exposure periods, dosages of fumigant applied, and temperatures during fumigation were varied. One-half of the seed samples received one fumigation; the others were fumigated twice. Two series of germination tests were made, the first within 5 days following fumigation of seeds and the second after 84 days of postfumigation storage at 50° F.

Under the conditions of these studies the germination of rice was not affected by fumigation with hydrocyanic acid. Injury resulting from methyl bromide fumigations could be identified as dead seed. Little evidence of retarded emergence of rice seedlings was observed. Results from germination tests illustrate the interdependence of dosage, temperature, period of exposure, and moisture content of seeds in contributing to seed injury from methyl bromide fumigation, and the importance of repeat fumigation as a dependent variable. Varietal differences and post-fumigation storage were the only variables considered that were found to be unimportant in contributing to seed injury from methyl bromide fumigations under the conditions of the study.

Investigations pertaining to the effect of fumigation on the germination of seeds were undertaken because of the possibility of injury to seeds in storage during fumigations for the eradication of the khapra beetle, *Trogoderma granarium* Everts. Studies with rice are an obvious extension of previous work reported by Strong & Lindgren (1959a, b, c).

The purpose of this paper is to evaluate several vari-

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ables which may influence the germination of rice seeds fumigated with methyl bromide or hydrocyanic acid. The results reported were obtained from tests made under controlled conditions and with proven techniques.

MATERIALS AND METHODS.—*Preparation of Seed Samples.*—Two varieties of rice, Calrose and Caloro, were used in all tests. The moisture content (m.c.) of seeds owing to normal fluctuations in storage ranged from 12.7 to 10.0% in Calrose and 13.8 to 10.7% in Caloro rice during the experimental period of approximately 12 months.

Obtaining rice with a graded moisture range of 8, 10, 12 and 14% required careful adjustment of moisture and conditioning of seeds. Apparent difficulties were involved, but persistent drying of seeds, addition of water, or alternation of drying and addition of moisture resulted in rice samples of the desired moisture content.

Seeds were dried in narrow wire-screen containers placed in a forced-air oven maintained at 125 to 130° F. The drying period ranged up to 3 hours at any one time, but never longer. The moisture content of seeds before drying and the desired moisture level indicated the length of time seeds were to be held in the air-oven. Water was added to increase the moisture content of the seeds. After drying or addition of water, seeds were conditioned in airtight polyethylene bags for 7 days at 50° F. Then seeds were blended and moisture tests were made to determine the necessity for additional moisture adjustments. The moisture content of seeds was determined by the air-oven method specified in the A.O.A.C. Official Methods of Analysis (Horwitz 1955).

When the moisture content of rice had reached the desired level, each lot of seeds was divided into samples of equal size, approximately 50 grams per sample, and put into small rough-woven cotton bags. Seeds were removed from these bags only for germination tests. Each cloth sample-bag of rice was placed in an airtight polyethylene bag to prevent moisture changes. Prepared samples awaiting fumigation were held in storage at 50° F.

Fumigation of Seeds.—Two variables considered in this study, varietal differences and moisture content of seeds, were treated in the preceding section on preparation of seed samples. Additional variables under consideration involved directly with the fumigation of seeds were: the exposure period, temperature during fumigation, dosage levels, and repeat fumigation.

Test samples were removed from storage and held at the desired fumigation temperature before removing airtight polyethylene bags. Seeds of all four moisture levels were placed in slatted crates inside the fumigation chambers to be fumigated together. The crates permitted thorough circulation of gases.

Seeds were fumigated in 100-cubic-foot gastight chambers with continuous circulation of air within the fumatorium. Temperatures were thermostatically controlled at 50, 70, and 90° ± 1° F., but no attempt was made to control humidity. Fumigations were replicated twice.

Two dosages of fumigant, one high and one low, were used throughout the study. Dosages were adjusted according to exposure periods and temperatures during fumigation. When the temperature was lowered, dosages usually were increased for an exposure period. Further, as the period of exposure was decreased, usually the dosages of fumigant were increased. An exposure period of 72 hours was selected for beginning tests with hydrocyanic acid, and an exposure period of 24 hours was selected for initial tests with methyl bromide. When seeds were injured at these exposures with the dosages applied, tests were made with shorter exposure periods.

Table 1.—Exposure periods, temperatures, and dosages of hydrocyanic acid and methyl bromide used for the fumigation of rice seeds.

Fumigant	Exposure (hours)	Temperature (°F.)	Dosage[a] Low	Dosage[a] High
Hydrocyanic acid	72	90	1.0	2.0
		70	1.0	2.5
		50	1.0	3.0
Methyl bromide	24	90	1.5	4.0
		70	2.0	4.0
		50	2.5	4.0
	8	90	2.0	5.0
		70	3.0	5.0
		50	3.0	6.0
	2	90	3.0	6.0
		70	3.0	6.0
		50	3.0	6.0

[a] Pounds per 1,000 cubic feet.

The exposure periods, temperatures during fumigation, and dosages of fumigants applied are given in table 1. Concentrations of fumigants were not determined for each dosage or exposure interval. However, typical curves for the fall in concentrations of methyl bromide were given previously by Strong & Lindgren (1959a).

All seeds received an initial fumigation. One-half of the samples were fumigated a second time 21 days after the initial exposure, to test the effect of repeat fumigation on the germination of rice. After fumigation, seeds were aerated for 24 hours before repackaging in airtight polyethylene bags. Undoubtedly slight moisture changes occurred during aeration of seeds; however, no attempt was made to adjust moisture after the initial fumigation. Seed samples were held in postfumigation storage at 50° F. and removed only for germination tests.

Germination Tests and Assessment of Results.—Two series of germination tests were made, the first within 5 days following fumigation of seeds, and the second after 84 days (12 weeks) in storage at 50° F. Seeds were germinated at alternating temperatures of 68 and 86° F. in upright rolled-paper-towel substrata.

Germination tests and seedling interpretations were made in accordance with regular methods of Official Seed Analysts (Assoc. Off. Seed Analysts 1954) except seeds with obvious mechanical injury were not planted, and 100 seeds were used per test for each fumigation replicate. Unfumigated seeds served as control standards for comparison.

The percentage of live seeds (normal and abnormal seedlings) and the percentage germination (normal seedlings) were the two criteria recorded for comparison of results. (See p. 139, USDA Agricultural Handbook No. 30, 1952, for a description of normal and abnormal rice seedlings.) The distinction made between live seeds and germinated seeds was considered important, since Cobb

(1956) found that injury from methyl bromide may be recognizable as retarded germination, as well as destruction of the germinative capacity of seeds.

RESULTS.—More than 2,300 germination tests of 100 seeds per test were made during this study, so it has been necessary to condense results materially and present only a summary of data essential to the understanding and discussion of variables investigated. The differences between percentages of germinated and live seeds were negligible, indicating little retarding of germination due to fumigation. Thus, results from fumigated seeds are presented only as the corrected percentages of reduction in germination. These results were calculated by extending Abbot's (1925) formula as follows: Corrected percentage reduction in germination =

$$\frac{\text{per cent germination in control samples} - \text{per cent germination in fumigated samples}}{\text{per cent germination in control samples}} \times 100$$

Hydrocyanic Acid Fumigation.—Exposure of two varieties of rice with a graded moisture range of 8, 10, 12, and 14% to hydrocyanic acid fumigation for 72 hours at temperatures and dosages listed in table 1 did not result in significant reductions in the germinative capacity of seeds. For this reason, only a summary of results for the extremes in treatments are presented in table 2.

Methyl Bromide Fumigation.—Results from the first series of germination tests (seeds planted within 5 days after fumigation) are presented in tables 3 to 5 as the corrected percentages of reduction in germination (given as nearest whole numbers). These data indicate that several of the variables considered in this study may have contributed to the reductions in germination resulting from fumigation with methyl bromide. This supposition is discussed more fully below.

DISCUSSION OF RESULTS FROM METHYL BROMIDE FUMIGATIONS.—Seven factors were considered of primary importance in this study. These factors are listed and discussed separately in regard to the importance of each variable in contributing to the injury of rice seeds from methyl bromide fumigation.

Varietal Differences.—Calrose rice seeds were of somewhat higher quality than Caloro, as indicated by results from germination tests with unfumigated control samples of the two varieties. However, by examination of the data presented in tables 3 to 5, it is obvious that seeds of the two varieties of rice respond quite similarly to methyl

Table 2.—Corrected percentages of reduction in the germination of two varieties of rice after two fumigations with hydrocyanic acid for 72 hours at three temperatures.

Variety of Rice	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosages in Pounds per 1,000 Cubic Feet: 90 F.—2.0	70 F.—2.5	50 F.—3.0
		Per Cent Reduction in Germination		
Calrose	8	0.0	1.0	2.0
	10	1.5	0.0	1.6
	12	0.0	0.0	0.0
	14	0.0	3.0	5.6
Caloro	8	0.0	0.0	2.1
	10	1.5	1.6	0.0
	12	1.0	1.0	2.1
	14	0.0	0.0	3.5

Table 3.—Corrected percentages of reduction in the germination of two varieties of rice after one and two methyl bromide fumigations for 24 hours at various temperatures and dosages.

No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosages in Pounds per 1,000 Cubic Feet: 90° F. 1.5	90° F. 4.0	70° F. 2.0	70° F. 4.0	50° F. 2.5	50° F. 4.0
		Per Cent Reduction in Germination					
		Variety Calrose					
1	8	2	5	3	5	1	2
	10	0	8	3	9	5	0
	12	1	28	5	29	11	7
	14	12	91	13	83	8	17
2	8	3	21	7	23	1	10
	10	8	17	10	23	8	8
	12	12	85	15	69	10	13
	14	16	96	51	97	27	43
		Variety Caloro					
1	8	0	0	3	6	1	5
	10	0	21	4	20	15	5
	12	10	27	9	28	11	7
	14	11	91	24	88	18	9
2	8	8	9	7	15	4	9
	10	7	69	8	47	8	20
	12	11	87	18	64	9	17
	14	24	99	56	92	25	32

Table 4.—Corrected percentages of reduction in the germination of two varieties of rice after one and two methyl bromide fumigations for 8 hours at various temperatures and dosages.

No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosages in Pounds per 1,000 cubic Feet: 90° F. 2.0	90° F. 5.0	70° F. 3.0	70° F. 5.0	50° F. 3.0	50° F. 6.0
		Per Cent Reduction in Germination					
		Variety Calrose					
1	8	0	0	2	0	0	6
	10	3	9	3	9	0	1
	12	3	4	11	10	3	5
	14	0	84	11	19	0	0
2	8	0	4	6	6	1	8
	10	4	8	3	5	6	7
	12	5	23	15	20	4	8
	14	15	88	29	76	0	19
		Variety Caloro					
1	8	0	5	0	1	2	2
	10	1	0	3	4	4	4
	12	0	10	3	12	9	4
	14	5	28	3	23	14	11
2	8	0	13	0	5	2	3
	10	8	13	7	8	5	12
	12	14	33	12	23	14	10
	14	23	89	12	41	14	23

Table 5.—Corrected percentages of reduction in the germination of two varieties of rice after one and two methyl bromide fumigations for 2 hours at various temperatures and dosages.

No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosages in Pounds per 1,000 Cubic Feet					
		90° F.		70° F.		50° F.	
		3.0	6.0	3.0	6.0	3.0	6.0
		Per Cent Reduction in Germination					
		Variety Calrose					
1	8	4	1	0	0	4	3
	10	7	2	0	3	0	0
	12	2	1	2	6	0	0
	14	4	8	8	6	1	2
2	8	8	8	0	1	1	0
	10	1	4	1	2	6	4
	12	7	8	9	8	0	2
	14	4	19	14	10	3	6
		Variety Caloro					
1	8	1	0	2	0	0	0
	10	3	4	0	0	0	0
	12	6	0	0	0	4	0
	14	5	1	3	6	7	1
2	8	0	6	1	1	0	0
	10	8	7	1	1	2	4
	12	10	8	6	8	4	5
	14	11	20	8	9	7	9

bromide fumigation. In this respect the results tend to agree with those reported by Lubatti & Blackith (1957) on germination tests following fumigation with methyl bromide by suggesting that the main differences in the reaction of cereals to methyl bromide lie between the species.

Moisture Content of Seeds.—Results from germination tests with rice seeds of a graded moisture range (8, 10, 12, and 14%) following fumigation with methyl bromide show increased percentages of reduction in germination with an increase in moisture content (tables 3 and 4). Examples of graded increases in percentages of reduction in germination that may be correlated directly with graded increases in moisture are found in tables 3 and 4 for the higher dosages of fumigant applied at 70 and 90° F. The critical moisture content appears to lie between 10 and 12%. It is evident from the data presented in tables 3 to 5, however, that in many instances seeds were not injured by fumigation with methyl bromide. This conclusion suggests that visible moisture effects may be a result of interactions with other variables. When discussing results from more extensive tests with wheat, Strong & Lindgren (1959a) regarded temperature, dosage, or repeat fumigation, alone or in combination, as important variables to consider in regard to their interactions with moisture to influence the germination of seeds fumigated with methyl bromide.

Period of Exposure to Fumigant.—A view of the results presented in tables 3 to 5 is sufficient to show that as periods of exposure are decreased, germination injury attributable to methyl bromide fumigation decreases.

Temperature During Fumigation.—In general, where rice seeds were affected by methyl bromide fumigation, increases in temperature resulted in increasing percentages of reduction in germination (tables 3 to 5). An effort was made, however, to allow an opportunity to isolate temperature from other variables. Differences between 50° F. and 70° F. are greater than those between 70° F. and 90° F. in the results presented in table 3 for fumigations with 4 pounds of methyl bromide per 1,000 cubic feet at each of three temperatures for 24 hours. These data are quite similar to those obtained by Strong & Lindgren with barley (1959b) and oats (1959c).

Dosage of Fumigant.—Eradication programs require somewhat higher than normal dosages of fumigant, so two dosage levels, one low and one high, were considered for each set of conditions found in this study. Paired comparisons may be made between dosage levels from the results presented in tables 3 to 5 by horizontal examination of the data, but the influence of dosage is best illustrated by results presented in table 3.

Repeat Fumigation.—High dosages of methyl bromide and long exposure periods are employed in eradication programs (for example, khapra beetle eradication) so that repeat fumigations are not required except in unusual situations. Seed sanitation, however, usually requires more than one fumigation to prevent injury from insects. Thus, the effect of repeat fumigations on germination of seeds is an important factor to consider. Results given in tables 3 to 5 suggest that repeat fumigation is a dependent variable, as increased injury occurred after the second fumigation only in cases where injury was indicated after one fumigation. Presumably, when seeds have a predisposition to injury after one fumigation, the injurious effect of methyl bromide is greatly enlarged by two fumigations.

Post-fumigation Storage.—A second series of germination tests was made after postfumigation storage of seeds for 84 days (12 weeks) at 50° F. Significant differences were not observed in the percentages of reduction in germination between tests made immediately (within 5 days) after fumigation and those made 84 days later. Results from the latter tests are not presented, as they would merely duplicate trends suggested by the data in tables 3 to 5. The results concurred with those found previously by Strong & Lindgren (1959a, b, c) in tests with other cereal grains, and the authors agree with Cobb (1958) in concluding that fumigation injury occurs not by delayed action but only when seeds are in contact with the fumigant.

References Cited

Abbot, W. S. 1925. A method of computing the effectiveness of an insecticide. Jour. Econ. Ent. **18**: 265–7.

Association of Official Seed Analysts. 1954. Rules for testing seeds adopted by Association of Official Seed Analysts, effective July 1, 1955. Proc. Assoc. Off. Seed Analysts, **44**.

Cobb, Rodney D. 1956. The effects of methyl bromide fumigation on seed germination. Proc. Assoc. Off. Seed Analysts **46**: 55–61.

Cobb, Rodney D. 1958. Seed germination after fumigation with methyl bromide for khapra beetle control. California Dept. Agric. Bull. **47**: 1–16.

Horwitz, William (Editor) 1955. Official methods of analysis of the Association of Official Agricultural Chemists, 8th ed. Washington, D. C., A.O.A.C.

Lubatti, O. G., and R. E. Blackith. 1957. Fumigation of agricultural products. XV.—Germination and growth of cereals fumigated with methyl bromide. Journ. Sci. Food Agric. 8: 691–7.

PMA and Bureau of Plant Industry, Soils, and Agricultural Engineering. 1952. Manual of testing agricultural and vegetable seeds. U. S. Dept. of Agric., Agric. Handbk. No. 30.

Strong, R. G., and D. L. Lindgren. 1959a. Effect of methyl bromide and hydrocyanic acid fumigation on the germination of wheat. Jour. Econ. Ent. 52(1): 51–60.

Strong, R. G., and D. L. Lindgren. 1959b. Effect of methyl bromide and hydrocyanic acid fumigation on the germination of barley. Jour. Econ. Ent. 52(2): 319–22.

Strong, R. G., and D. L. Lindgren. 1959c. Effect of methyl bromide and hydrocyanic acid fumigation on the germination of oats. Jour. Econ. Ent. 52(3): 415–8.

A Larval Diet for the Boll Weevil Containing an Acetone Powder of Cotton Squares[1]

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ABSTRACT

A larval diet for the boll weevil, *Anthonomus grandis* Boh., has been developed using a protein source natural to the species—an acetone powder from squares, bolls, or leaves from the cotton plant. The most successful diet contains acetone powder from squares in addition to agar, salts, sugar, vitamins, cholesterol, soybean protein and water. The weevils reared on this diet are larger than those reared on diets using soybean protein or casein as the only source of protein. By adding two mold inhibitors, sorbic acid and methyl *p*-hydroxybenzoate, the need for the sterilization of the diet is eliminated. Successive generations of weevils can be maintained in the laboratory by allowing adults to feed and to oviposit in cotton squares. Eggs are then recovered from the squares and are placed in the larval diet. The weevils reach maturity in 2 to 3 weeks at 27° C.

A satisfactory larval diet for the boll weevil, *Anthonomus grandis* Boh., has been developed by Vanderzant & Davich (1958). Following the practices of Bottger (1942) and Beck (1950), this diet utilizes an agar-cellulose mixture as a supporting medium. The essential ingredients are soybean protein, cysteine, sucrose, salts, B vitamins, cholesterol, cellulose and agar. The diet is autoclaved and the eggs are transferred under aseptic conditions. Preliminary studies had shown soybean protein to be superior to casein, although the latter will permit the development of some larvae.

The soybean diet is suitable for many purposes; however, a rearing medium was desired which contained protein less foreign to the species—namely, protein from the cotton plant itself. Such a medium was needed for studies of the effect of larval nutrition on diapause in the adult.

Perhaps the easiest way to obtain a crude protein fraction is by the preparation of an acetone powder. In preliminary tests at Baton Rouge, mixtures using an acetone powder of squares as the protein source appeared promising, and eventually a satisfactory diet was developed which is now used routinely at this laboratory.

PREPARATION.—The acetone powder is prepared in a blender by grinding squares (with or without bracts) or sliced bolls with three to five volumes of acetone. Cotton leaves may also be used. The solids are recovered by filtration and are resuspended twice more with acetone in the blender to obtain a fine, almost white powder. About 1 gram is obtained from every 5 grams of squares or leaves, or from 10 grams of bolls. The powder is mixed with other dietary constituents in the following proportions:

	Grams
Acetone powder of squares, bolls, or leaves	10–20
Sucrose	2.0
Cholesterol	0.05
Wesson's salts (Nutritional Biochemicals Corp.)	0.3
Choline chloride	0.1
Agar (Difco Bacto)	3.0
Sorbic acid, in 1 ml. of ethanol (Nutritional Biochemicals Corp.)	0.1
Methyl *p*-hydroxybenzoate, in 0.5 ml. of ethanol (Goldschmidt Chemical Corp.)	0.1
Distilled water	100
B vitamins, in 1 ml. of water (Vanderzant 1957) or (Beck 1953)	

The agar is first dissolved on a water bath, and the vitamin solutions are then added, followed by the sucrose, cholesterol, and Wesson's salts, which have been ground together in a mortar. The two mold inhibitors sorbic acid and methyl *p*-hydroxybenzoate may be added at this point as 10% and 20% ethanolic solutions, respectively, to give a final concentration of about 0.1% of each. The need for sterilization of the diet by autoclaving is eliminated when these two materials are added. The same inhibitors, incidentally, are essentially without effect in preventing microbial contamination of the diet based on soybean protein. The acetone powder is stirred in last with an electric stirrer.

Owing to its thick consistency, the mixture is best dispensed with a cake decorator using a section of an oil can spout in place of the regular tip. The diet is placed in the bottom of 45-×15-mm. vials to a depth of about 6 mm. The vials may be capped individually with aluminum foil or screw caps, or a large group of about 50 vials may be wrapped in a plastic material such as Saran wrap.

Best results are obtained when the vials have been heated in an oven overnight at 100° C. and when the weevil eggs have been disinfected. A brief wash with a 0.02% solution of mercuric chloride in a 1:3 ethanol-water mixture followed by two washes with sterile water is adequate. The eggs are placed in the diet by means of a dissecting needle that has been flattened at the tip. The weevils mature in 2 to 3 weeks at 30°C. To collect eggs, adults are held in screen cages and are provided with cotton squares that are kept fresh in a dish of moist sand. Illumination from a 100-watt mercury vapor lamp has been found to help initiate egg production (Earle *et al.* 1958).

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Table 1.—Development of two strains of boll weevils on modified larval diets.[a]

Source of Acetone Powder	Strain of Eggs	Number of Eggs	Yield of Adults (%)	Average Adult Weight (mg.)	Weevils with Soft Cuticles After 2 Weeks (%)
		Boll extract in place of sucrose and salts			
Bolls	Louisiana	44	66	9.5	17
	Mexico	53	89	13.9	7
Squares	Louisiana	48	86	9.0	6
	Mexico	49	72	11.0	3
		Sucrose and Wesson's salts			
Bolls	Louisiana	48	75	11.6	18
	Mexico	48	77	13.8	3
Squares	Louisiana	45	51	8.8	8
	Mexico	46	78	11.6	6

[a] Eggs obtained from square-reared Louisiana weevils or third generation, medium-reared Mexico weevils. Weevils reared at 21° C. for 3 weeks, then at 27° C. until adult emergence. Acetone powder from field squares and bolls picked on August 11.

Adults reared on acetone powder diets appear normal and are nearly as large as those that develop from squares in the field. More than 5,000 adults have already been reared on this diet, and 4 cultures have been maintained in the laboratory for 4 generations.

MODIFICATIONS OF DIET.—Various water extracts of macerated bolls were substituted for the sucrose and salt mixture. While a water extract of bolls was being processed, it was noted that the addition of acetone caused a brown gummy material to precipitate. Several extracts of bolls purified by this method were tested as substitutes for the sucrose and Wesson's salt mixture with eggs from two different cultures—square-reared Louisiana weevils and third-generation medium-reared weevils that had originated in Mexico. Table 1 gives the results with one of these extracts prepared from young bolls collected in the field on August 11. Ten grams of powder were used in these diets. The boiled aqueous extract was mixed with 15 volumes of acetone and filtered. For each gram of bolls there remained 24 mg. of solutes.

Larger weevils are obtained by allowing the larvae to develop at low temperatures, as shown by the data in table 2. These weevils were reared on a medium containing 10 grams of acetone powder of squares obtained from greenhouse cotton. In diets with acetone powder from bolls or leaves, the larvae develop more slowly and the adults are slightly smaller.

Omission of sucrose from the diet appeared to have little effect on development. Omission of salts and sucrose allowed only about 10% of the larvae to reach the adult stage. Substitution of corn oil for sucrose and salts allowed more than 50% of the larvae to reach maturity,

Table 2.—Influence of temperature during larval stages on size of adults reared from medium containing acetone powder of squares picked from greenhouse cotton on April 29.

Temperature (° C.)	Number of Weevils	Average Adult Weight (mg.)
	Mexico Strain	
21[a]	20	14.6
30	8	12.3
	Louisiana Strain	
21[a]	23	13.2
30	21	10.6

[a] Transferred to 27° C. after 3 weeks in order to accelerate pupal development.

Table 3.—Influence of protein concentration on size of adults from a first-generation laboratory strain of Louisiana weevils reared at 27° C.

Acetone Powder (gm.)[a]	Protein Added	Number of Weevils	Average Weight (mg.)
10	—	48	10.5
15	—	93	11.9
20	—	50	11.7
10	2 gms. soybean protein	49	12.3
10	2 gms. peanut flour	23	11.3

[a] Acetone powder of squares collected from field in October.

but the adults were slightly undersized. These results indicate that the acetone powder probably supplies enough carbohydrate but is deficient in some of the salts. There is little difference in the weights of weevils reared from diets containing 0.5, 1.0, 2.0, or 4.0 grams of sugar. Weevils did develop more slowly on the diet containing the highest concentration, however,

Table 1 shows that the diets containing the boll extract are just as good as those with sucrose and Wesson's salts. In this experiment the boll powder appeared better than the square powder when the percentages of eggs reaching maturity and the average weights were considered alone. However, in one respect the boll powder appeared to be inferior—the cuticles remained soft in a higher percentage of the adults, particularly among the Louisiana weevils.

The protein content determined by Kjeldahl analysis of acetone powder of squares varies between 20% and 22%. Boll and young leaf powder contains about 18%, and mature leaf powder about 24%. Diets with 10 grams of square powder per 100 ml. of water therefore contain about 1.8% protein. Weevils will develop normally on this diet even though the protein concentration is only about half that found in fresh squares. Larger weevils can be reared from diets containing more protein, as the data in table 3 indicate. The diet containing a mixture of square powder and soybean protein appears to be a very satisfactory one. It permits the development of larger weevils without the use of greater amounts of acetone powder.

There is some indication that the nutritional value varies among samples of acetone powder. Weevils reared on media containing powder from squares picked from greenhouse cotton on April 29 (table 2) were slightly larger than those reared from squares grown in the field and picked on August 11th (table 1). However, this difference could have been due partly to the strain of weevils.

Several differences were noted between the two weevil strains, and these probably represent adaptations by the strain from Mexico that had been reared on artificial media for three generations. In general, a higher percentage of weevils from the laboratory Mexico strain reached maturity, the adults weighed more, and more developed normal cuticles, (table 1). The differences may not be due entirely to adaptation, since in another experiment first generation Mexico weevils also grew to a larger size on an artificial diet than first generation Louisiana weevils (table 2).

REFERENCES CITED

Beck, S. D. 1950. An aseptic method for rearing European corn borer larvae. Jour. Econ. Ent. **43**(1): 4–6.

Beck, S. D. 1953. Nutrition of the European corn borer, *Pyrausta nubilalis* (Hbn.) III. An unidentified growth factor required for larval growth. Jour. Gen. Physiol. **36**: 317–25.

Bottger, G. T. 1942. Development of synthetic food media for use in nutritional studies of the European corn borer. Jour. Agric. Res. **65**: 493–500.

Earle, N. W., L. D. Newsom, M. S. Blum, J. S. Roussel, and R. C. Gaines. 1958. Initiation and termination of diapause in the boll weevil *Anthonomus grandis* (Boh.). Presented at the 6th annual meeting of the Ent. Soc. America, December 1958.

Vanderzant, Erma S. 1957. Growth and reproduction of the pink bollworm on an amino acid medium. Jour. Econ. Ent. **50**: 219–21.

Vanderzant, Erma S., and T. B. Davich. 1958. Laboratory rearing of the boll weevil: A satisfactory larval diet and oviposition studies. Jour. Econ. Ent. **51**(3): 288–91.

Thimet Soil Treatments for Control of Leaf Miners, Galls, and Lace Bugs[1]

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ABSTRACT

Application of Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate) granules to the soil around birch, holly, and boxwood in 1957 controlled all season the birch leaf miner (*Fenusa pusilla* (Lep.)), the holly leaf miner (*Phytomyza ilicis* (Curt.)), and the boxwood leaf miner (*Monarthropalpus buxi* (Lab.)) on the treated trees. In 1958 the higher dosages used in each experiment continued to control leaf miners.

Treatments of Thimet on soil gave good control of the first brood of honey locust pod gall maker (*Dasyneura gleditschiae* (O.S.)), but were ineffective for later broods. Similar treatments of andromeda and azalea plants controlled lace bugs.

Control of birch, holly, and boxwood leaf miners by applications of Thimet (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate) granules was reported by Schread (1958). The effect of these treatments has been followed further. In addition tests were made for the control of the honey locust pod gall maker (*Dasyneura gleditschiae* (O.S.)), which causes the honey locust pod gall, andromeda lace bug (*Stephanitis globulifera* (Mat.)), and azalea lace bug (*Stephanitis pyrioides* Scott).

BIRCH LEAF MINER (*Fenusa pusilla* (LEP.)).—Birch trees treated in 1957 were examined again in 1958. Fifty leaves taken at random were examined June 11, 1958 (first brood) and July 7 (second brood). The results were as follows:

Date of Treatment, 1957	*Thimet per Acre (lb.)*	*Per Cent Kill, 1958*	
		First Brood	*Second Brood*
June 4	2.5	—	1.0
June 4	5.0	33.0	1.0
June 4	10.0	100.0	0.0
June 4	20.0	100.0	1.0
Apr. 12	22.0	—	10.0
Apr. 12	40.0	—	83.0
Untreated (No. of larvae)		244	471

It is obvious that the Thimet persisted in the birch trees for more than a year. The 10 and 20 pounds per acre rates applied June 4, 1957, still killed all the first generation of leaf miners in 1958. The average number of eggs per treated leaf was 5.6 and per untreated leaf 4.9. Control of the second generation in 1958 was much lower. Only the rate of 40 pounds per acre killed a substantial percentage of the leaf miners.

HOLLY LEAF MINER (*Phytomyza ilicis* (CURT.)).—Data relative to the control of the holly leaf miner during the summer of 1958 with Thimet 2% granules applied July 19, 1957, are given in table 1. The two lowest treatments of 4 and 8 ounces of formulation used per tree provided almost no control whereas 12 and 16 ounces gave good to complete control, respectively. Although no noticeable phytotoxicity developed during the growing season of 1957 as a result of the treatments made on July 19 of that year, mild yellowing of the new foliage growing on the tree treated with 16 ounces of 2% Thimet granules became noticeable as the leaves expanded in 1958. The trees that received lesser amounts of the granules were not visibly affected by the treatments. Green pigment slowly replaced the yellow so that by autumn the holly foliage had a normal green color. An average of 9 square feet of ground was treated under each tree.

BOXWOOD LEAF MINER (*Monarthropalpus buxi* (LAB.)).—Control of the boxwood leaf miner on August 15, 1958, with 2% Thimet granules used July 18, 1957, was complete with 0.16, 0.24 and 0.32 ounces of actual Thimet per 3-foot boxwood plant having a circumference of 5 to 6 feet, and covering 2.5 square feet of ground. An 0.08 ounce treatment gave negligible results.

HONEY LOCUST POD GALL MAKER. (*Dasyneura gleditschiae*. (O.S.)).—Thimet 2% granules and 48% emulsions were used in experiments intended to determine their effectiveness in controlling the honey locust pod gall maker. One gallon of drench was applied with a 2-gallon watering can to the ground under Moraine locust trees at the dilutions given in table 2. Two per cent granules were distributed with a 13-mesh wire sieve at the rate of 1.5 and 3 pounds of formulation per tree to give amounts of actual Thimet shown in table 2. Trees averaged 8 feet in height, and each treatment was duplicated. Treatments were applied evenly to the surface of the ground from the base to the drip line of each tree covering an average of 20 square feet per tree. None was worked or watered into the sod in which the tree were growing. There were four untreated check trees.

Control data were obtained by taking random samples of 10 compound leaves from each replicate of treatment and from the untreated checks. All galled and uninjured leaflets were counted to obtain percentages of injury

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Table 1.—Control of holly leaf miner Sept. 3, 1958, with 2% Thimet granules applied July 19, 1957.

Ounces of 2% Granules Thimet per Tree	Average No. Miners per Leaf[a]	Per Cent Kill
4	2.7	14.5
8	7.0	22.1
12	3.0	71.6
16	2.0	100.0
Untreated	3.8	

[a] Sampled 20 leaves per treatment.

(table 2). The nature of Thimet formulation appeared to have no significant influence on control of the honey locust pod gall maker when treatments were applied to the soil. Control, however, improved with the successive increase of actual Thimet applied.

Control of the first brood of gall insects in May was significantly higher than that obtained for the second brood in June. Although data concerning the additional broods occurring during the remainder of the summer are not given here, the results were about the same as those obtained in June.

ANDROMEDA LACE BUG (*Stephanitis globulifera* (Mat.)) *and* AZALEA LACE BUG (*S. pyrioides* SCOTT).—To control lace bugs 8% Thimet granules were diluted with 2 pounds of dry sand per treatment and applied by hand at dilutions of actual Thimet given in tables 3 and 4 to 3 square feet of soil under 42″ andromeda (*Pieris japonica* (Thomb)) and 1.75 square feet of soil under 20″ azaleas (*Azalea hinodegiri*). The Thimet was raked into the top 1 inch of soil from the base to the drip line of each plant. Four gallons of water were used to soak each plot when the treatment was made and three times thereafter at about 3-day intervals. Each treatment was replicated twice with two untreated plants of both species used as checks.

Table 2.—Effect on honey locust pod gall of Thimet soil treatments made April 10, 1957.

Formulation Used	Ounces of Actual Thimet per Tree[a]	Per Cent Leaflets[b] Galled	
		May 29	June 20
Soil drench, 48% E	0.62	19.2	53.6
	.31	27.9	80.9
Granules, 2%	0.96	13.8	74.2
	.48	25.8	67.9
Untreated		79.8	96.1

[a] An average of 20 square feet of ground treated per tree
[b] Twenty compound leaves examined per treatment.

Table 3.—Control of andromeda lace bug, August 5, with Thimet granules applied July 17, 1958.

Ounces of Actual Thimet	Second Brood Adults		Per Cent Control
	Dead	Alive	
0.16	100	46	68.4
.32	316	55	85.1
Untreated	0	279	

Table 4.—Control of azalea lace bug August 1, with Thimet 8% granules applied July 9, 1958.

Ounces of Actual Thimet	Living Adults and Nymphs[a]
0.08	7
.16	13
.32	0
Untreated	81

[a] Nymphs made up 95% of the population.

Data on control were taken by counting all the dead and living adult lace bugs that could be seen in 5 minutes of examination of each plant.

Soil treatments of 8% Thimet granules gave good control of second brood adult andromeda lace bug (table 3). Innumerable dead nymphs were present, but none were counted. No live nymphs were seen. Examinations made on August 29 indicated that no live adults or nymphs were present on the treated andromeda. On the untreated check plants 29 living adults were found. On September 4, seven living third-brood nymphs and 10 second-brood adults were counted on the check plants whereas none were seen on the treated ones. Seven live nymphs and two adults were counted on September 15 on the untreated, and no live ones on the treated plants. Fifty live fourth-brood nymphs and 62 third-brood adults were counted on the untreated andromeda on October 9. On this date there were no live nymphs or adults on the treated plants.

Data given in table 4 indicate good control of azalea lace bug with Thimet granules. Dead nymphs present on the treated azaleas were too numerous to count, hence no record was made of their number. Examinations made on August 15 indicated 18 living lace bugs on the untreated plants, but none on the treated ones. A survey made 1 month later (September 15) indicated seven live adults and no live nymphs present on the check plants. On this date no living ones were found on the treated azaleas.

REFERENCE CITED

Schread, John C. 1958. Thimet soil treatments for the control of leaf miners. Jour. Econ. Ent. 51(2): 245–6.

Seed Treatment with Systemic Insecticides for Control of Spotted Alfalfa Aphids under Kansas Conditions[1]

JACK L. BISHOP and C. C. BURKHARDT[2]

ABSTRACT

Seed was treated with demeton at the concentration-time combinations of 0.25% for ½ hour soaking, 0.5% for ½ and 1 hour soaking. Ronnel, Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) were tumbled with seed as dry treatments at the rates of 1, 2, and 4 pounds of toxicant per 100 pounds of seed.

No significant reduction occurred in germination or emergence when seed was treated with Thimet or Di-Syston. Slight emergence reduction occurred in seed treated with Ronnel. Both germination and emergence were reduced after 6 months of storage of seeds treated with demeton, probably resulting from inadequate drying of seed following soak treatment.

Seed was planted immediately, 1 week, 1 month, 6 months, and 1 year after treatment. Treatments showed no reduction in aphicidal effectiveness after storage. No visible phytotoxic effects were observed in any of the treated alfalfa except for a temporary browning of the leaf margins of alfalfa treated with ronnel.

The best over-all results were obtained using Di-Syston at the rates of 2 and 4 pounds toxicant per 100 pounds of seed. These rates gave 100% control for the entire 6-week period. The 1-pound rate of Di-Syston gave above 95% control throughout the period. Thimet at 4 pounds gave 100% control through the fourth week and good control for the remainder of the 6-week period. The 2 pound rate proved to be 100% effective through the fourth week, 85% effective through the fifth week, and over 75% effective through the sixth week. The 1-pound rate gave satisfactory control only through the fourth week.

Statistical analyses indicated no significant differences occurred between any rates used in Di-Syston or between the 4-pound rate of Thimet and any rate of Di-Syston. Ronnel and demeton were generally ineffective beyond the second week.

The spotted alfalfa aphid, *Therioaphis maculata* (Buckton), has become of major economic importance during its brief history in the United States. Since its establishment in New Mexico, probably in 1953 (Dobson & Watts 1957), it had spread from coast to coast and from central Wisconsin on the north, to deep into Mexico on the south by 1957 (Stern & Reynolds 1957, Peters & Painter 1958). It has become one of the major pests of alfalfa in the United States, causing losses of more than $81 million to alfalfa growers in 1954, 1955, and 1956 (U.S.D.A. 1957).

The first appearance of the spotted alfalfa aphid in Kansas was in August 1954, and its subsequent spread was reported by Harvey & Hackerott (1956) and Sorensen *et al.* (1958). In 1955 and 1956 this aphid caused an estimated loss of $2.4 million and $8.5 million, respectively, to Kansas alfalfa growers.

The aphid is extremely damaging to young alfalfa seedling plants. Reynolds *et al.* (1957) reported that treatment is justified in California when aphid populations average one aphid per seedling plant, and Dobson & Watts (1957) reported that an average of one aphid per seedling plant in New Mexico severely reduced stands.

For protection of seedling plants from aphid attacks, conventional spraying or dusting has presented several disadvantages. Movement of heavy machinery over seedling plants damages the stand. The small plants afford little surface for receiving and retaining the insecticides. Rapid growth of the plants causes a dilution of the insecticide concentration producing ineffective dosages (Dobson & Watts 1957, Reynolds *et al.* 1957). Furthermore, many of the natural enemies of the aphid are destroyed by the use of contact insecticides.

Because of promising results reported in early preliminary experiments by workers in Southwestern States as well as by Burkhardt (1957) in Kansas, the work reported here was undertaken. It was believed that there might be potential benefits in the use of systemic insecticides as seed treatments of Kansas alfalfa under Kansas evironmental conditions.

MATERIALS AND METHODS.—Seed from two alfalfa varieties, Buffalo and Kansas Common, were used. Charcoal-impregnated formulations of thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate) 44% by weight, Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) 50%, and 25% ronnel W.P., containing methyl cellulose 2% weight as a sticker, were applied as dry treatments at the rates of 1, 2, and 4 pounds of actual toxicant per 100 pounds of seed. One-fourth pound of seed was placed in each wide-mouth Mason quart jar along with the appropriate dosage of insecticide mixture. The jars were rotated on a tumbling machine for 2½ hours, a period previously determined as adequate for complete seed coverage. The seeds were then stored in the jars in which they were treated. Seeds also were soaked in a 0.25% emulsion of demeton ½ hour, and a 0.5% emulsion for ½ hour, and for 1 hour. One-fourth pound of seed was placed in each of three cloth bags which were immersed in the 0.25% and 0.5% demeton-water emulsions contained in wide-mouth gallon jars. The jars were shaken constantly to maintain a homogeneous emulsion throughout the soaking period. After soaking, seeds were spread evenly over clean paper and left 8 hours for drying, aided by an electric fan blowing over the seed.

Untreated seed and seed treated with only methyl cellulose were used as controls.

Tests for germination, soil-flat emergence, aphicidal

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activity or effectiveness, and phytotoxicity were made at the following time intervals after treatment: 0 day (immediately), 1 week, 1 month, 6 months, and 12 months. The intervals were chosen to study possible effects of these materials during storage of treated seed. Seeds were sent to the Kansas State Seed Laboratory at Topeka, where germination tests were conducted on blotters in constant temperature boxes. Soil-flat emergence tests were conducted at Manhattan by planting seeds in sterilized soil in flats watered from below by watering pans. Emergence counts were made 7 and 10 days after planting. To evaluate aphicidal effectiveness of these treatments, aphids were introduced on the alfalfa seedlings 2 days after emergence. Third instar nymphs were used in preference to adults or last instar nymphs, to allow more time before reproduction occurrred. Twenty nymphs were placed on alfalfa and were confined by inverting a pint paper carton over the infested plants. The paper carton had been previously prepared by removing the bottom and gluing a nylon mesh fabric over the opening for ventilation and light. Daily counts were made of the live aphids on the alfalfa under the paper cartons. The use of a dental mirror with an angled shaft facilitated counting the aphids, especially those on the under surface of the leaves. The number of dead aphids was obtained by subtracting the live aphids counted from 20, the number of aphids originally introduced. At the end of each 7-day period the live aphids, if any, were removed by sweeping the plants with a small brush. Twenty more nymphs were introduced to fresh alfalfa in a different part of the flat, enclosed in the carton, and left for a new 7-day testing period. Six consecutive 7-day testing periods were conducted in each flat or treatment.

Table 1.—Number of alfalfa seed germinated after 7 days as determined by the Kansas State Seed Laboratory, Topeka. 1957–58.

Variety and Treatment[a]	Pounds of Toxicant/ 100 Lbs. of Seed[b]	Per Cent Germinated When Planting Followed Treatment as Shown				
		0 Day	1 Wk.	1 Mo.	6 Mos.	1 Yr.
Kansas Common						
Thimet	1	92	87	91	90	91
	2	89	89	89	90	87
	4	90	92	89	82	91
Demeton	0.25%—½	91	92	84	4	6
	0.5%—½	89	91	91	22	10
	0.5%—1	86	90	89	2	4
Di-Syston	1	85	88	94	92	91
	2	89	88	90	92	91
	4	91	85	87	91	92
Ronnel	1	91	94	94	86	93
	2	93	90	93	89	92
	4	88	87	88	89	91
Untreated[c]		88	89	91	87	94
Methyl cellulose[d]		90	89	87	87	94
Buffalo						
Thimet	1	80	79	84	84	85
	2	77	79	77	82	83
	4	77	81	78	81	84
Demeton[b]	0.25%—½	79	69	81	8	8
	0.5%—½	73	73	79	15	13
	0.5%—1	76	80	86	6	7
Di-Syston	1	76	82	81	78	80
	2	82	76	78	81	80
	4	78	83	80	71	84
Ronnel	1	80	81	83	86	82
	2	86	77	84	83	80
	4	82	76	81	76	82
Untreated[c]		81	77	83	80	83
Methyl cellulose[d]		88	75	81	80	89

[a] Averages from 3 replications of 100 seeds each.
[b] Demeton treatments and rates are 0.25% soak for ½ hour, 0.5% for ½ and 1 hour. Demeton-treated seed was reported as very moldy after 6 months or 1 year.
[c] Averages from 10 replications of 100 seeds each.
[d] Averages from 2 replications of 100 seeds each.

Table 2.—Emergence percentages 10 days following planting treated alfalfa seed in soil flats. Manhattan, Kans., 1957–58.

Variety and Treatment[a]	Pounds of Toxicant/ 100 Lbs. of Seed[b]	Per Cent Emerged When Planting Followed Treatments as Shown:				
		0 Day	1 Wk.	1 Mo.	6 Mos.	1 Yr.
Kansas Common						
Thimet	1	89	84	83	89	93
	2	85	80	88	90	93
	4	83	79	88	89	91
Demeton	0.25%—½	91	80	97	5	3
	0.5%—½	88	54	82	8	6
	0.5%—1	92	66	87	4	2
Di-Syston	1	87	83	85	88	90
	2	85	82	83	91	95
	4	94	81	78	93	95
Ronnel	1	74	81	83	87	91
	2	71	70	79	84	89
	4	69	65	74	77	79
Untreated[c]		87	76	95	90	91
Methyl cellulose[c]		89	73	91	88	85
Buffalo						
Thimet	1	74	71	84	81	78
	2	76	76	76	77	77
	4	71	74	81	77	73
Demeton	0.25%—½	81	84	85	5	5
	0.5%—½	76	76	89	7	3
	0.5%—1	70	71	85	9	6
Di-Syston	1	84	74	78	75	73
	2	76	77	80	79	79
	4	87	70	79	80	78
Ronnel	1	69	58	82	77	73
	2	66	74	83	72	67
	4	63	66	82	72	67
Untreated[c]		76	80	81	80	75
Methyl cellulose[c]		75	64	85	79	76

[a] Based upon 150 seeds.
[b] Demeton-treatment rates were: 0.25% and ½ hour soak, 0.5% and ½ and 1 hour soaks. State Seed Laboratory reported seeds moldy after 6 months or 1 year.
[c] Averages of two flats of 150 seeds per flat.

Effects of phytotoxicity were analyzed by gross observation of the plants, and by dry-weight "root growth-top growth" ratios. Random samples (15 individuals) of the plants were washed out of the flats, cleaned, and the root system severed from the top growth. These samples were placed in seamless tin containers, dried in a constant-temperature oven at 105° C. until a constant weight was obtained, and weighed.

RESULTS.—*Germination.*—Data on alfalfa seed germination are presented in table 1. In general little or no reduction in germination resulted from seed treatments. Demeton-treated seed which was stored 6 months or longer showed extensive reduction of germination, probably resulting from inadequate drying of seed following the soak treatment. This seed was reported by the State Seed Laboratory as being definitely moldy. Except for the demeton treatments, storage for as much as 1 year did not reduce germination significantly. Untreated as well as treated seed of Kansas Common germinated

better than the Buffalo variety. Methyl cellulose at 2% by weight showed no harmful effects on seed germination.

Soil-Flat Emergence Counts and Plant Growth Observations.—Data on soil-flat emergence of seeds are shown in table 2. Ronnel appeared to reduce emergence consider-

Table 3.—Aphicidal effectiveness of systemic insecticides applied to alfalfa seeds planted at varying intervals after treatment. Manhattan, Kans., 1957–58.

Variety and Treatment[a]	Pounds of Toxicant/ 100 Lbs. of Seed[b]	Per Cent Aphids Dead Following Each of 6 Successive Weeks					
		1st	2nd	3rd	4th	5th	6th
Planted immediately after treatment							
Kansas Common Thimet	1	100	100	90	75	70	90
	2	100	100	80	100	95	60
	4	100	100	100	100	100	70
Demeton	0.25%—½	90	35	15	30	10	20
	0.5%—½	100	15	45	30	20	20
	0.5%—1	100	25	60	60	10	30
Di-Syston	1	100	100	100	100	80	75
	2	100	100	100	100	100	80
	4	100	100	100	100	100	100
Ronnel	1	100	60	15	50	45	20
	2	100	60	20	30	60	25
	4	100	75	100	100	100	50
Untreated		50[b]	0	5	5	30	15
Methyl cellulose		5[b]	5	0	15	10	15
Buffalo Thimet	1	100	100	90	75	50	40
	2	100	100	100	95	100	65
	4	100	100	100	100	75	95
Demeton	0.25%—½	85	45	20	65	15	25
	0.5%—½	100	90	45	40	35	10
	0.5%—1	100	55	75	45	30	15
Di-Syston	1	100	100	95	90	90	70
	2	100	100	100	100	100	100
	4	100	100	100	100	100	100
Ronnell	1	100	45	20	35	40	30
	2	100	65	25	80	85	35
	4	100	100	90	90	100	75
Untreated		70[b]	5	5	25	25	5
Methyl cellulose		60[b]	10	10	0	15	5
Planted 1 week after treatment							
Kansas Common Thimet	1	100	100	55	75	55	50
	2	100	100	100	90	60	65
	4	100	100	95	100	65	80
Demeton	0.25%—½	95	10	20	15	15	15
	0.5%—½	90	60	50	15	5	20
	0.5%—1	100	80	60	20	20	25
Di-Syston	1	100	100	100	100	95	100
	2	100	100	100	100	100	100
	4	100	100	100	100	100	100
Ronnel	1	100	90	75	45	55	10
	2	100	90	80	65	70	30
	4	100	100	90	85	65	35
Untreated		5	5	30	5	15	10
Methyl cellulose		20	20	5	10	15	5
Buffalo Thimet	1	100	100	50	80	55	40
	2	100	100	85	100	60	50
	4	100	100	90	70	80	75
Demeton	0.25%—½	65	50	50	30	20	10
	0.5%—½	90	55	60	30	10	20
	0.5%—1	100	100	75	35	20	15
Di-Syston	1	100	100	70	70	95	100
	2	100	100	100	100	100	100
	4	100	100	100	100	100	100
Ronnel	1	100	90	40	45	45	15
	2	100	90	90	60	25	25
	4	100	100	95	75	50	40
Untreated		45	15	25	15	10	5
Methyl cellulose		5	25	20	5	35	10
Planted 1 month after treatment							
Kansas Common Thimet	1	100	100	100	100	85	65
	2	100	100	100	100	95	80
	4	100	100	100	100	100	100
Demeton	0.25%—½	65	60	40	30	25	15
	0.5%—½	60	65	60	50	40	20
	0.5%—1	90	80	75	65	60	25
Di-Syston	1	100	100	100	100	100	100
	2	100	100	100	100	100	100
	4	100	100	100	100	100	100
Ronnel	1	95	70	55	45	40	20
	2	100	100	60	55	45	30
	4	100	100	80	85	70	40
Untreated		0	20	20	10	10	5
Methyl cellulose		10	20	25	10	10	10
Buffalo Thimet	1	100	100	100	90	80	60
	2	100	100	100	100	95	80
	4	100	100	100	100	100	95
Demeton	0.25%—½	45	50	65	40	30	15
	0.5%—½	75	50	60	45	35	20
	0.5%—1	100	80	95	70	45	20
Di-Syston	1	100	100	100	100	100	100
	2	100	100	100	100	100	100
	4	100	100	100	100	100	100
Ronnel	1	100	80	55	40	25	15
	2	100	75	55	50	40	35
	4	100	100	80	70	65	50
Untreated		10	10	10	5	5	10
Methyl cellulose		0	20	20	10	5	5
Planted 6 months after treatment							
Kansas Common Thimet	1	100	100	100	100	90	100
	2	100	100	100	100	100	100
	4	100	100	100	100	95	100
Demeton	0.25%—½	100	100	65	30	20	15
	0.5%—½	100	100	95	50	40	20
	0.5%—1	100	100	70	80	55	35
Di-Syston	1	100	100	100	100	100	100
	2	100	100	100	100	100	100
	4	100	100	100	100	100	100
Ronnel	1	100	80	35	50	25	20
	2	100	100	100	100	100	90
	4	100	100	100	100	100	100
Untreated		10	0	0	0	0	0
Methyl cellulose		5	0	0	0	0	0
Buffalo Thimet	1	100	100	100	100	100	80
	2	100	100	100	100	100	100
	4	100	100	100	100	100	100
Demeton	0.25%—½	100	100	100	30	25	10
	0.5%—½	100	100	95	80	40	20
	0.5%—1	100	100	85	45	60	40
Di-Syston	1	100	100	100	100	100	100
	2	100	100	100	100	100	100
	4	100	100	100	100	100	100
Ronnel	1	100	80	30	35	35	25
	2	100	100	100	100	100	90
	4	100	100	100	100	100	100
Untreated		0	5	10	0	5	0
Methyl cellulose		0	0	10	5	5	0

[a] Treatment rates for demeton were: 0.25% as ½ hour soak, 0.5% as ½ hour and 1 hour soaks.

[b] A fungus disease infected these aphids.

Table 4.—Means of square roots of numbers of aphids dead, observed on alfalfa treated with systemic insecticides as seed treatments. Manhattan, Kans., 1957–58.

Treatment	Pounds of Toxicant/ 100 Lbs. of Seed[a]	Square Root Means of Dead Aphids After the Indicated Weeks Following Emergence of Alfalfa					
		1st	2nd	3rd	4th	5th	6th
Thimet	1	4.44	4.33 *[b]	3.90	3.91	3.55	3.31
	2	4.48	4.40	4.35	4.28	4.00	3.61
	4	4.48	4.49	4.40	4.32	4.11	4.06
Demeton	0.25%—½	3.75 *	3.12	2.94 *	2.43 *	1.92	1.79
	0.5%—½	4.02 *	3.39	3.38 *	2.74 *	2.23	1.96
	0.5%—1	4.33 *	3.62	3.52 *	3.02 *	2.58	2.23
Di-Syston	1	4.54	4.47	4.42	4.31	4.25	4.15
	2	4.55	4.55	4.56	4.52	4.47	4.31
	4	4.57	4.55	4.57	4.55	4.48	4.49
Ronnel	1	4.49 *	3.63 *	2.78	2.66	2.56 *	1.96
	2	4.54 *	3.95 *	3.33	3.35	3.38 *	2.74
	4	4.55	4.38	4.14	3.89	3.81 *	3.08
Untreated		2.09 *	1.48	1.75	1.45	1.64	1.38
Methyl cellulose		1.65	1.69	1.65	1.41	1.69	1.33

L.S.D. at 5% level = 0.43

[a] Treatments for demeton were 0.25% as a ½ hour soak, and 0.5% as ½ hour and 1 hour soaks.
[b] Asterisks indicate significant difference at the 5% level between the figures in the columns on either side of the asterisks. Means paralleled by the same vertical line are not significantly different at P = .05.

ably, especially at the rates of 2 and 4 pounds of toxicant per 100 pounds of seed. Emergence as well as germination was drastically reduced in demeton-treated seeds stored for 6 months or longer, probably for the reason explained above. No other treatment had harmful effects on emergence, nor was there any indication that storage reduced emergence to any extent.

A study of plant growth subsequent to emergence revealed no further visible phytotoxicity in any treatments except ronnel, which showed browning of the margins of the leaves, but this effect disappeared when the seedlings became about 4 weeks old. Gross observations of treated and untreated plants indicated that the treatments were apparently somewhat stimulative to plant growth under greenhouse conditions.

Aphicidal tests.—Data pertaining to effects of the insecticidal treatments on aphid populations are presented in table 3. Table 3 gives data on the effectiveness of the

Table 5.—Means of square roots of numbers of aphids dead after 6-week period for the various planting times on alfalfa treated with systemic insecticides as seed treatments. Manhattan, Kans., 1957–58.

Treatment	Pounds of Toxicant/ 100 Lbs. of Seeds[a]	Means of Square Roots of the Numbers of Dead Aphids for Each of the Following Planting Times After Treatment				
		0 Day	1 Wk.	1 Mo.	6 Mos.	1 Yr.
Thimet	1	3.68	3.60 *[b]	3.91 *	4.42	4.13
	2	4.08	3.93	4.21 *	4.53	4.24
	4	4.27	4.06 *	4.37	4.54	4.37
Demeton	0.25%—½	2.62	2.39	2.36 *	3.26	—[c]
	0.5%—½	2.88	2.62	2.73 *	3.57	—[c]
	0.5%—1	2.95	2.98	3.18 *	3.75	—[c]
Di-Syston	1	4.24	4.20	4.40	4.57	4.38
	2	4.42	4.50	4.47	4.58	4.51
	4	4.50	4.53	4.53	4.58	4.56
Ronnel	1	2.92	3.21 *	2.83	3.10 *	2.51
	2	3.18	3.38	3.13 *	4.50 *	3.11
	4	3.97	3.74	3.66 *	4.54 *	3.64
Untreated		1.98	1.84 *	1.53 *	1.17	1.13
Methyl cellulose		1.67	1.83 *	1.63 *	1.15	1.26

L.S.D. at 5% level = 0.30

[a] See footnote a, table 4.
[b] See footnote b, table 4.
[c] Seeds did not emerge.

treatments applied to seeds planted immediately after treatment. The testing period extended from June 20 to August 7, 1957. The plants attained an average height of 7 inches. A fungus disease was reponsible for the high percentage of dead aphids in the untreated and methylcellulose treated seedlings during the first week of the period. Observation disclosed no fungus disease in aphids of the treated alfalfa.

No difference was observed in the effectiveness of the treatments between the two alfalfa varieties used. Demeton and ronnel were effective for the first week, but thereafter provided little protection against the aphids. Thimet proved to be very effective through the fourth week and supplied fair protection through the sixth week. Di-Syston resulted in a high degree of effectiveness throughout the 6-week period.

Table 3 presents data on aphicidal effectiveness on seedlings from seeds planted 1 week after treatment. The test period was from June 28 to August 14, during which time the plants reached an average height of 8 inches. The results obtained compared favorably with those from the first test.

Information on aphicide testing of seeds planted 1 month after treatment is given in table 3. The test period started October 13 and continued through November 28, at which time the average plants were 6 inches tall. Even though temperatures were consistently lower during this period than those of the two earlier testing periods, the data indicate practically no variation from those presented in sections 1 and 2 of table 3.

Results of aphicidal effectiveness from seeds planted 6 months after treatment are also presented in table 3.

These tests were conducted in a plastic greenhouse. The results obtained indicated that effectiveness of the treatments was better than it was in the three earlier tests. The greatest improvement was shown in the 2- and 4-pound rates of ronnel, which remained effective for the entire test period. Thimet and Di-Syston at all rates were highly effective for the 6-week period. All aphids were dead by the first or second day of each week for all rates of Di-Syston. Demeton performed only slightly better than it had in previous tests.

Aphicide tests involving seed planted 1 year after treatment showed results similar to the 6-months-after-treatment test except they were less effective. Di-Syston again gave effective control for the entire 6-week period and Thimet provided excellent control through the fourth week but lost much of its effectiveness after that. Ronnel was ineffective after the second week in this test.

The data obtained from the aphicide tests were treated statistically following a square root transformation of the data. The number of aphids dead on each day of each week of the 6-week period was recorded, with 20 the largest possible number. The square roots of these numbers were taken and the means of the square roots treated statistically. Since it was desirable that the treatment be effective for a period of 6 weeks following emergence of the seedlings, the data were treated to indicate the effects of 6 weeks' time upon the effectiveness of the treatments (table 4). Since no significant differences existed between varieties, they were not analyzed separately. Table 4 shows that Thimet and Di-Syston retained their effectiveness throughout the 6-week periods and that the 4-pound rate of Thimet and all rates of Di-Syston were not significantly different as aphicides in these tests.

Table 6.—Array of means of dry weight ratios of top growth to root growth of alfalfa treated with systemic insecticides as seed treatments. Manhattan, Kans., 1957–58.

Treatment[a]	Pounds of toxicant/100 lbs. of seed[a]	Means in array[b]
Ronnel	4 lbs.	1.87
Di-Syston	2 lbs.	1.86
Di-Syston	4 lbs.	1.86
Untreated		1.82
Ronnel	2 lbs.	1.79
Methyl cellulose		1.75
Demeton	0.5%—½	1.69
Di-Syston	1 lb.	1.68
Thimet	2 lbs.	1.65
Demeton	0.25%—½	1.64
Thimet	1 lb.	1.64
Demeton	0.5%—1	1.62
Thimet	4 lbs.	1.59
Ronnel	1 lb.	1.59
L.S.D. at 5% level = 0.19		

[a] See footnote a, table 4.
[b] Means paralleled by the same vertical line are not significantly different at P = .05.

Table 5 presents results of statistical treatment of data recorded, showing the effects of storage on the effectiveness of the insecticides. The data indicate that storage did not significantly alter the effectiveness of any of the three rates of Di-Syston, and generally the 4-pound rate of Thimet was not affected by storage. Both tables 4 and 5 show that Thimet at 4 pounds and all rates of Di-Syston were highly effective since the figure 4.58 indicates 100% control.

Observations on the Pea Aphid.—At one time treated alfalfa in the greenhouse became accidentally infested with pea aphids, *Macrosiphum pisi* (Harris). The treatments seemed to have little effect on these pea aphids because they multiplied and attained heavy populations, even killing the alfalfa treated with ronnel, demeton, and the lighter rates of Thimet and Di-Syston.

Gross Root Structure Studies.—Root growths showed as much variation among the individuals within the same treatment as among various treatments, and as much among the untreated plants as between the untreated group and the treated groups.

Top Growth-Root Growth Ratios.—Table 6 lists the array of means of the ratio of top growth to root growth. No treatment had a significantly larger mean ratio than the untreated. No difference occurred between varieties, hence they were treated together. Only demeton, 0.5%–1; Thimet, 4 pounds; and ronnel, 1 pound showed a significantly lower ratio than the untreated.

References Cited

Burkhardt, C. C. 1957. Chemical control of the spotted alfalfa aphid. Proc. 12th Ann. North Central Branch, Ent. Soc. America 12: 72–73.

Dobson, R. O., and J. G. Watts. 1957. Spotted alfalfa aphid occurrence on seedling alfalfa as influenced by systemic insecticides and varieties. Jour. Econ. Ent. 50(2): 132–5.

Harvey, T. L., and H. L. Hackerott. 1956. Apparent resistance to the spotted alfalfa aphid selected from seedlings of susceptible alfalfa varieties. Jour. Econ. Ent. 49(3): 289–91.

Peters, D. C., and R. H. Painter. 1958. Studies on the biologies of three related legume aphids in relation to their host plants. Kansas Agric. Expt. Sta. Tech. Bull. 93, 44 pp.

Reynolds, H. T., T. R. Fukuto, R. L. Metcalf and R. B. March. 1957. Seed treatment of field crops with systemic insecticides. Jour. Econ. Ent. 50(5): 527–39.

Sorensen, E. L., C. C. Burkhardt and Wayne Fowler. 1958. Alfalfa seed production in Kansas. Kansas Agric. Expt. Sta. Circ. 290, 22 pp.

Stern, Vernon H., and H. T. Reynolds. 1957. Developments in chemical control of the spotted alfalfa aphid in California, 1955–56. Jour. Econ. Ent. 50(6): 817–21.

United States Department of Agriculture. 1957. The spotted alfalfa aphid. U.S. Dept. Agric., Agric. Res. Serv., Leaflet 422, 8 pp.

Toxicity of House Fly Larvae to Insecticides Administered as Single Oral Dosages to Chicks[1,2]

MARTIN SHERMAN and ERNEST ROSS[3]

ABSTRACT

The acute toxicity of Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), Dow ET-14 (*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate), Dow ET-15 (*O*-methyl *O*-(2,4,5-trichlorophenyl)phosphoramidothioate), malathion, and phenothiazine to 1-week-old chicks and the toxic effect of the droppings from these treated chicks on 1-day-old house fly (*Musca domestica* (L.)) larvae were investigated. The LD-50, 7 days after treatment, in terms of mg. of technical toxicant per kg. of chick weight follows: Diazinon, 8.4; Dipterex, 65; malathion, 370; Dow ET-14, 890; and Dow ET-15, 1,180. Phenothiazine was nontoxic at the highest level that it was possible to feed accurately to the chicks (1,970 mg./kg.). The effect of treatment on chick weight gain was also studied.

The degree of fecal toxicity to house fly larvae was directly related to the dosage of insecticide ingested by the chick and inversely related to the period of time after ingestion that the feces were voided. Feces collected daily from chicks fed nonlethal levels of insecticides caused greater than 90% mortality in house fly larvae for varying periods of time, as follows: malathion, 1 day; Dipterex, 2 days; Dow ET-14, 3 days; and Dow ET-15, 6 days. Feces from Diazinon- and phenothiazine-treated chicks caused relatively low mortality in the larvae.

The treatment of poultry manure to control house fly (*Musca domestica* (L.)) larvae is generally effective for a comparatively short period (Hoffman & Monroe 1956, 1957; Wilson & Gahan 1957). The major factors involved in the failure of larvicidal sprays are discussed by Sampson (1956). The use of animals as vehicles for the distribution of insecticides in manure would offset some of the difficulties inherent in the conventional methods of application, especially that of penetration.

The control of dipterous larvae in the manure of warm blooded animals that had been orally dosed with insecticides has been shown by several investigators (Knipling 1938, Bruce 1942, Eddy *et al.* 1954, Robbins *et al.* 1957). Previous studies with cattle and other animals (Robbins *et al.* 1956, 1957, Plapp & Casida 1956) indicate that the bulk of ingested insecticides and their metabolites are excreted in the urine and relatively small amounts in the feces. This fact means that relatively little unchanged insecticide or its toxic metabolites are available in the feces for larvicidal activity. This shortcoming is overcome with poultry, however, since the fowl voids both urine and feces through a common opening, the cloaca. Therefore, most of the insecticides and their metabolites subsequently find their way into the droppings and are available for larvicidal activity.

This study was undertaken to investigate the acute toxicity of several insecticides to 8- and 9-day-old New Hampshire chicks and to determine the effect of the droppings from these chicks on house fly larvae.

MATERIALS AND METHODS.—The composition of the insecticides tested was as follows: Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), technical, 89% with 11% related compounds; Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), technical, 100%; Dow ET-14 (*O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate), wettable powder at 25% used as a proprietary formulation of Korlan®); Dow-ET-15 (*O*-methyl *O*-(2,4,5-trichlorophenyl)phosphoramidothioate), technical; malathion, technical 95%; and phenothiazine, wettable powder at 98.5%. Standard stock solutions of the technical grade materials and suspensions of the wettable powders were made in acetone and distilled water, respectively.

Five hundred and sixty-eight New Hampshire straight-run chicks, 8 to 9 days of age, were used in this study. The chicks were weighed just prior to treatment and these weights were used to determine the amount of toxicant each chick received. However, feed and water were withheld from the chicks for 16 hours prior to the weighing so that variation owing to intake of food would be minimized. Dosage was calculated on the basis of milligrams

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of technical toxicant per kilogram of body weight.

Administration of the required dosage was accomplished in two ways: 1) Where an acetone solution of the technical grade of insecticide was used, the proper volume of stock solution was introduced into a gelatin capsule by means of a micropipette. In a previous study (Sherman & Rosenberg 1953), it was shown that these capsules were not harmful to chicks. The acetone was allowed to evaporate before the capsule was sealed and force-fed to the chick. 2) The stock suspensions of wettable powders were diluted to the proper concentration and the required volume was introduced directly into the crop by means of a 5-ml. pipette.

The chicks were housed in electrically heated battery brooders in groups of 10 individuals, each of which received the same treatment. Feed and water were supplied *ad libitum*. The feces[4] were collected in dropping pans under each treatment pen. The chicks were observed for 1 week after treatment to record symptoms of intoxication and to determine mortality. At the end of the 1-week period, the survivors were weighed to determine whether there was a sublethal effect that manifested itself as a subnormal weight gain or actual weight loss.

The toxicity of the chick feces to 1-day-old larvae of the house fly was determined. The feces were collected daily during the week following treatment. The dropping pans were carefully scraped so as to avoid contaminating the droppings from one day's collection with that from the next. The feces from the chicks receiving the same treatment were mixed thoroughly and frozen until tested. The fecal sample to be analyzed for toxicity was placed in a Stender dish, allowed to defrost, and brought to the required moisture content by the addition of approximately 2 ml. of distilled water. Ten newly hatched house fly larvae were placed in each fecal sample. The dish was then covered with a fine muslin cloth to prevent larval escape or oviposition by volunteer muscoid flies and stored for 3 days at a temperature of 25° C. Two days after the introduction of the larvae, an additional 2 ml. of water was added to each dish to replace loss by evaporation. Three days after the introduction of the larvae into the fecal sample, the number of survivors was determined. Each test was replicated at least three times at weekly intervals.

EXPERIMENTAL RESULTS AND DISCUSSION.—A total of 50 chicks were kept as untreated controls. None of these chicks died or showed any deleterious symptoms.

Acute Toxicity to Chicks.—The acute toxicity of Diazinon, Dipterex, Dow ET-14, Dow ET-15, malathion, and phenothiazine are given in table 1.

The LD-50 of Diazinon was calculated to be 8.4 mg./kg. Although the birds were observed for 1 week, all deaths occurred within 24 hours and usually within 2 to 3 hours after ingestion of the capsule containing the insecticide. Chicks that were affected by this substance salivated excessively and went into convulsions. Between convulsive spasms the chicks were ataxic and appeared to have some paresis. After death, there was opisthotonos with hyperextension of the legs. Autopsy showed peripheral hemorrhages, which caused the flesh to appear purplish in color. The gizzard showed contraction and its lining was removed with difficulty.

The LD-50 of Dipterex was calculated to be 65 mg./kg.

Table 1.—Acute toxicity of single doses of several insecticides to New Hampshire chicks 7 days after treatment.

Dosage[a]	No. of Chicks	Per Cent Mortality	Mean Weight-Gain Ratio
Diazinon, technical			
15.0	10	90	—
12.5	20	80	1.99
9.0	30	73	2.13
8.0	10	60	—
7.0	20	25	2.10
5.0	10	0	2.13
2.7	10	0	2.26
1.5	10	0	2.22
Untreated	30	0	2.21
Dipterex, technical			
150	10	100	—
125	20	100	—
90	30	77	1.98[b]
80	20	85	1.91[b]
50	20	15	2.05[b]
27	20	0	2.12
15	10	0	2.17
Untreated	18	0	2.19
Dow ET-14 (25%) wettable powder			
1600	10	100	—
1200	10	80	1.17[c]
1000	10	90	1.23[c]
800	10	30	1.45[c]
500	10	0	1.67[c]
Untreated	10	0	1.87
Dow ET-15, technical			
1600	10	90	1.30[c]
1200	10	50	1.35[c]
1000	10	30	1.54[c]
800	10	0	1.52[c]
500	10	0	1.65[c]
Untreated	10	0	1.87
Malathion, technical			
450	20	90	1.46[c]
400	10	60	1.85[c]
350	10	40	2.10
300	20	20	2.07[b]
200	20	0	2.11
125	10	0	2.13
Untreated	20	0	2.23
Phenothiazine (98.5%) wettable powder			
1970	10	0	1.82
1478	10	0	1.82
985	10	0	1.89
493	10	0	1.86
99	10	0	1.93
Untreated	10	0	1.87

[a] Milligrams of technical insecticide per kilogram of body weight.
[b] Differs significantly from the untreated control at P = 0.05 level.
[c] Differs significantly from the untreated control at P = 0.01 level.

All deaths occurred within 13 hours with the great majority occurring 2 to 3 hours after treatment. Affected chicks salivated excessively but were quiet and not convulsive. Dyspnea was evident. Autopsy revealed congestion of the lungs and hemorrhages into the body cavity. The gizzard muscle was relaxed and the lining was easily removed.

The LD-50 of Dow ET-14 was calculated to be 890 mg./kg. This is considerably lower than the LD-50 of 6,500 mg./kg. reported by The Dow Chemical Co. (Anonymous 1957). Death occurred over an extended period

[4] Inasmuch as the feces and urine are voided through a common vent, the term feces as used in this paper refers to the mixture of the two.

ranging from less than 24 hours to as long as 72 hours. All the birds became drowsy within 1 hour after ingesting this material and showed very little activity, appearing anesthetized. They slept much of the time and showed little interest in feeding or drinking. During this time the wings were held away from the body in much the same manner as chicks suffering from heat, which would indicate a high body temperature. These symptoms lasted for several days but by the end of the week the surviving chicks appeared normal. Autopsy showed intestinal and peripheral hemorrhages. The flesh was purplish in color. The kidneys were pale and the lungs were congested. The gizzard muscle was relaxed. The odor of this insecticide was present in the flesh and was particularly evident upon initially opening the body cavity.

The LD-50 of Dow ET-15 was calculated to be 1,180 mg./kg. Death occurred within 24 to 36 hours. The symptoms exhibited by the chicks treated with this material were similar to but more intense than those exhibited by the chicks treated with Dow ET-14. Autopsy also showed a similar picture. The odor associated with this material was especially pronounced in the body.

The LD-50 of malathion was calculated to be 370 mg./kg. Death occurred within 24 hours, usually within the first hours after treatment. The affected chicks showed ataxic gait and had a tendency to stagger and fall. Neither convulsions nor excitement was evident. Post-mortem examination showed congestion of the lungs, hemorrhages in the kidney and spleen, and contraction of the gizzard muscle.

Phenothiazine was nontoxic at the maximum level that could be force-fed to the chicks without loss of test material. No pathological symptoms were observed.

Effect of Treatment on Weight-Gain.—Since the effect of these toxicants did not always result in death, the weight-gain ratio was used as a measure of subclinical toxicity. The weight-gain ratio was calculated by dividing the weight of the chick 1 week after treatment by the initial weight of the chick at the time of treatment. The mean weight-gain ratios of the survivors of the treated chicks were compared with those of their respective untreated controls and analyzed by the analysis of variance. This information is summarized in table 1. Dow ET-14 and Dow ET-15 affected the growth of the chicks so that they had a significantly smaller mean weight-gain ratio than their untreated controls. The fact that these materials had an anesthetizing effect on the chicks that slowed down their activity could be expected to show up in a subsequently lower weight-gain. Even those dosages that showed no lethal effect caused sufficient lethargy to reduce food intake and subsequent weight-gain. The retarding effect of Dow ET-14 on weight-gain was greater than comparable dosages of Dow ET-15.

Those dosages of Dipterex and malathion which caused chick mortality also caused a significant reduction in weight-gain among the survivors. Diazinon, however, caused no reduction in weight-gain among survivors, even at those concentrations which were highly lethal. Phenothiazine did not affect the weight-gain ratio of the chicks.

Toxicity of Feces to House Fly Larvae.—Figure 1 summarizes the toxicity of feces from insecticide-treated chicks to first-instar house fly larvae. Corrections for natural mortality were made by the use of Abbott's Formula (Abbott 1925). Mortality of house fly larvae in the manure from the untreated control chicks was very low, ranging from 0 to 12%.

The degree of fecal toxicity to larvae was directly correlated with the dosage of insecticide ingested by the chick and inversely correlated with the period of time after ingestion that the feces were voided.

Only feces voided within the first 24 hours from Diazinon-treated chicks were toxic to the house fly larvae. Moreover, feces from chicks receiving a dosage of 1.5 mg./kg. were nontoxic to the larvae. Robbins *et al.* (1957) studied the metabolism of this material in a cow and bioassayed the feces using horn fly (*Siphona irritans* (L.)) and house fly larvae. They administered orally by capsule a dosage of 20 mg./kg. They found that horn fly development and emergence was prevented in the feces sampled 6, 12, 18, 24, 36, and 48 hours after treatment of the cow. House fly emergence was prevented by the 48-hour fecal sample.

When chicks were treated with Dipterex at 90 and 50 mg./kg. there was 100% mortality of the house fly larvae placed in the fecal samples collected 3 days after treatment. However these dosages resulted in 77 and 15% mortality to the chicks. The dosages which were non-toxic to the chicks (*i.e.*, 27 and 15 mg./kg) resulted in fecal samples which gave 100% mortality of the larvae for 2 days and 1 day after treatment, respectively. Robbins *et al.* (1956) also studied the metabolism of this material in a cow and found that it was more rapidly metabolized and excreted in the urine than was Diazinon. They found that 66% of Dipterex and/or its metabolites were accounted for in 12 hours. Less than 3% of the total dose was excreted in the feces.

Dow ET-14 and ET-15 were tolerated by the chicks at comparatively high dosages and excreted in a toxic form so that the droppings were highly toxic to the house fly larvae for 2 to 7 days after the chick treatment. At comparable dosages Dow ET-15 was less toxic to the chicks and the droppings more toxic to the house fly larvae than Dow ET-14. At the highest dosage level at which these materials were nonlethal to the chicks, the feces caused more than 90% mortality to the larvae for 6 days after treatment with Dow ET-15 and 3 days after treatment with Dow ET-14.

Plapp and Casida (1958) found that Trolene®, a refined form of Dow ET-14 (Dow ET-57), was much more rapidly metabolized and excreted in the rat than in the cow. Trolene, administered to a cow at 100 mg./kg. was excreted unchanged in the feces at a level of 24 p. p. m. at 24 hours, 2.3 p. p. m. after 70 hours, and 0.1 p. p. m. after 120 hours. Trolene metabolites in the urine accounted for at least 49% of the administered dose.

Malathion, when fed at nontoxic levels to the chicks, was excreted in amounts highly toxic to the maggots for only 1 day with the mortality in the feces dropping rapidly on the second day after treatment. March *et al.*, (1956) studied the metabolism of malathion in hens and found that 60% of the consumed malathion had been excreted after 2 to 4 days. However, 97 to 98% of this excreted malathion was in the form of ionic water-soluble metabolites and degradation products. It was postulated that a large amount of the consumed malathion was me-

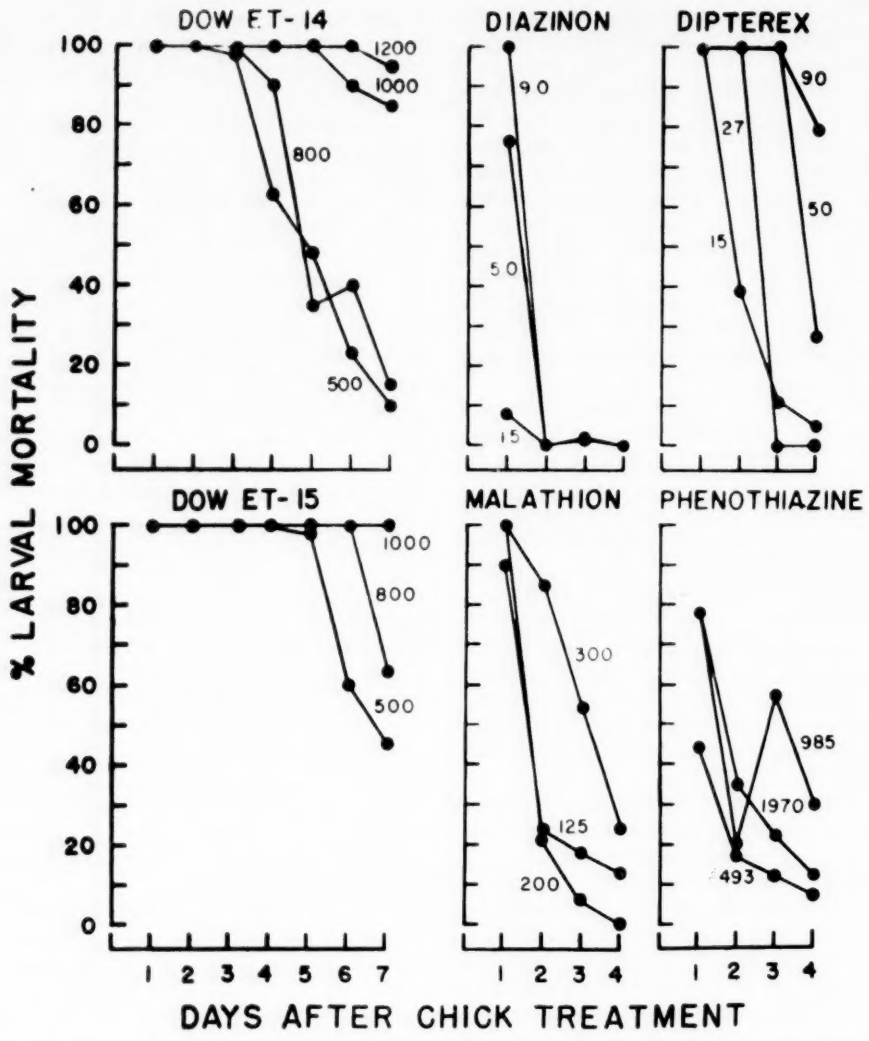

FIG. 1.—Toxicity of poultry manure from chicks treated with a single dose of insecticide to first-instar house fly larvae. Manure collected daily after chick treatment. Temperature 25° C. Figures within graphs refer to dosage (mg./kg.) of insecticide received by chick.

tabolized in the gut and directly excreted without being absorbed.

Phenothiazine, although completely innocuous to the chicks, caused only a low level of larval toxicity in the droppings.

REFERENCES CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Jour. Econ. Ent. **18**(2): 265–7.

Anonymous. 1957. Korlan an organic phosphate pesticide with low toxicity to warm blooded animals and a broad spectrum of insecticidal activity. ACD Information Bull. 109. 16 pp. The Dow Chemical Co., Midland, Michigan.

Bruce, W. G. 1942. Zinc oxide: a new larvicide for use in the medication of cattle for the control of horn flies. Kansas Ent. Soc. Jour. **15**: 105–7.

Eddy, G. W., W. S. McGregor, D. E. Hopkins, J. M. Dreiss, and R. D. Radeliff. 1954. Effects on some insects of the blood and manure of cattle fed certain chlorinated hydrocarbon insecticides. Jour. Econ. Ent. **47**(1): 35–38.

Hoffman, R. A., and R. E. Monroe. 1956. Control of house fly larvae in poultry droppings. Jour. Econ. Ent. **49**(5): 704–5.

Hoffman, R. A., and R. E. Monroe. 1957. Further tests on the control of fly larvae in poultry and cattle manure. Jour. Econ. Ent. **50**(4): 515.

Knipling, E. F. 1938. Internal treatment of animals with phenothiazine to prevent development of horn fly larvae in manure. Jour. Econ. Ent. **31**(2): 315–6.

March, R. B., T. R. Fukuto, R. L. Metcalf, and M. G. Maxon. 1956. Fate of P^{32} labeled malathion in the laying hen, white mouse, and American cockroach. Jour. Econ. Ent. **49**(2): 185–95.

Plapp, F. W., and J. E. Casida. 1958. Animal metabolism of insecticides, bovine metabolism of organophosphorous insecticides. Metabolic fate of *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate in rats and a cow. Jour. Agric. Food Chem. **6**(9): 662–7.

Robbins, W. E., T. L. Hopkins, and G. W. Eddy. 1956.

The metabolism of P^{32} labeled Bayer L 13/59 in a cow. Jour. Econ. Ent. 49(6): 801–5.

Robbins, W. E., T. L. Hopkins, and G. W. Eddy. 1957. Metabolism of insecticides, metabolism and excretion of phosphorus -32-labeled Diazinon in a cow. Jour. Agric. Food Chem. 5(7): 509–13.

Sampson, W. W. 1956. Insecticides used to control house fly larvae. Jour. Econ. Ent. 49(1): 74–77.

Sherman, Martin, and M. M. Rosenberg. 1953. Acute toxicity of four chlorinated dimethanonaphthalene insecticides to chicks. Jour. Econ. Ent. 46(6): 1067–70.

Wilson, H. G., and J. B. Gahan. 1957. Control of house fly larvae in poultry houses. Jour. Econ. Ent. 50(5): 613–4.

Locale of Aged Tedion Residues on Citrus Fruits[1]

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ABSTRACT

Most residues of organic pesticides on citrus fruit quickly penetrate the waxy cuticle and enter the oily portions of the peel, where they are protected from the various chemical and physical forces of weathering and are generally inaccessible to the pest organism. By using a laving technique with selective solvents on field-treated fruit, about 90% of the Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical) residues on oranges remained within the waxy cuticle, although not uniformly throughout. About 56% of the residue was coextracted with about 2% wax by laving the field-treated fruit with acetonitrile. About 60% of the residual Tedion on field-treated lemons was removed during commercial washing operations.

Carman & Gunther (1951) have proposed that aging residues of persisting organic insecticides applied to plant parts distribute themselves and their metabolites within cuticular and subcuticular tissues of the plant parts, largely through processes of solution-diffusion in the waxy and oily components of the tissue matrix. This penetration of citrus fruits, for example, results from the migration of the insecticide through the waxy cuticle into the oil glands, which then act as storage reservoirs for these lipoid-soluble materials. In support of this proposed penetration route, laboratory-prepared citrus oils from treated fruit have been found to contain high concentrations of some insecticides. Also, Gunther & Blinn (1955) have shown this migration graphically by use of their so-called A, B, C technique, in which A represents the extra-surface residue or residue adhering to the wax layer, B represents the cuticular residue or residue imbedded within or dissolved in the wax layer, and C represents the subcuticular residue or residue penetrated below the wax layer. The A, B, C residual behavior of ovex and dieldrin on lemons was presented graphically by Gunther & Blinn (1955) to show migration through the cuticle into the subcuticular portion of the peel. This migration is also demonstrated by the data for Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol) residues on and in both unwashed and detergent-washed oranges (Gunther *et al.* 1957).

The ovicide-larvicide Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical) is promising for the control of tetranychid mites on citrus; its effectiveness in the field is long-lived (Jeppson 1958). Residue studies of Tedion on citrus fruits by several analytical procedures indicate little if any loss of residual Tedion even after 100 days (Gunther 1958, Cassil & Fullmer 1958). In fact, detergent washing of fruit (rubbing fruit lightly in detergent solution) 2 days after treatment removed only minor quantities of residual Tedion (Gunther 1958), in striking contrast with the residual behavior of any of the other pesticides studied (Gunther & Blinn 1955, Gunther *et al.* 1957). It was therefore of interest to determine both the locale of aged Tedion residues in citrus fruits and the effect of commercial washing operations on any surface residues present.

MATERIALS AND METHODS.—*Selective-Solvent Laving.*—Mature Valencia orange trees were treated on April 26, 1958, with 1 pound of a wettable-powder formulation of 25% Tedion per 100 gallons of water. Application was made as a full-coverage spray, using a high-pressure reciprocating pump and manually operated spray guns.

After 23 days and again after 38 days, two mature fruits were picked from each quadrant of each of eight trees in the plot. Randomly selected 4-fruit subsamples were weighed and laved (the surface of the fruit thoroughly rinsed with the solvent) with the volumes of chloroform, n-hexane, or acetonitrile listed in table 1. The lavings were allowed to percolate through anhydrous sodium sulfate into a Kuderna-Danish evaporative concentrator. The solvent was evaporated to dryness, and the residue was dried to constant weight at 80° C. to obtain weight of wax and other material extractable under these conditions. Then 8-fruit samples were laved with the same volume per fruit of solvent, the solvents evaporated to dryness, and evaporation residues were analyzed for Tedion contents by the infrared procedure (Gunther *et al.* 1959). The laved fruits were then peeled, and 1-pound subsamples of minced peel were processed in the manner and with the equipment previously described (Gunther & Blinn 1955) to afford stripping solutions. Aliquots of these stripping solutions were analyzed for Tedion content by the infrared procedure.

Commerical Washing.—Mature lemon trees were treated on May 14, 1958, with 1 pound of a wettable-powder formulation of 25% Tedion per 100 gallons of water, as mentioned heretofore.

After 33 days 2 mature fruits were picked from each

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[3] Niagara Chemical Division, Food Machinery and Chemical Corp., Richmond, California.

[4] The authors wish to thank C. W. Wilson, of Sunkist Growers, Inc., Ontario, California, for commercial washing of the fruit; and M. J. Jesser and J. O. Complin of the University of California Citrus Experiment Station for assistance with field treatment.

Table 1.—Results of selective-solvent laving of Valencia oranges.

Solvent	Days After Treatment	Ml. Solvent per Fruit	P.p.m. Tedion in		Per Cent	
			Laving	Peel	Tedion in Laving	Wax and Extractives in Laving[a]
Chloroform	23	40	1.30	0.20	87	100[b]
	38	40	1.21	0.18	87	100[b]
n-Hexane	23	70	1.39	0.39	78	22[c]
	38	40	1.16	0.51	70	20[c]
Acetonitrile	23	20	0.74	0.61	55	2[d]
	38	40	1.12	0.84	57	4[d]

[a] Arbitrarily considering the chloroform laving as 100%.
[b] Hard, yellow wax.
[c] Hard, colorless wax.
[d] Soft, colorless wax.

quadrant, from each of 8 trees in the plot, and the resulting 64-fruit sample was processed as a unit. Replicate 64-fruit samples were processed separately.

All fruits in one replicate were peeled, and a 1-pound subsample of the minced peel was processed as described above. Aliquots of the final stripping solution were analyzed for Tedion content by the infrared procedure.

All fruits in the other replicate were put through a commercial washing operation before processing and were then analyzed as described above. This operation consisted of soaking the fruits for 2 minutes in a solution of 0.25% soap, 4% borax, and 2% boric acid at 115° F., then scrubbing with mechanical brushes for 1½ minutes in the same solution. The fruits were then rinsed in clear water and air-dried (Wilson 1958).

Discussion of Results.—The amounts of Tedion and wax found in the lavings and the amount of Tedion remaining in the laved peel of field-treated Valencia oranges are shown in table 1.

The solvents used for the surface laving of the fruit (table 1) were chosen for their gradient ability to dissolve plant waxes. Thus, as is shown in the table, chloroform was the most efficient solvent, n-hexane was of intermediate solvent power, and acetonitrile dissolved very little of the plant waxes. In this way it was possible to remove selectively different portions of the waxy cuticle, and determine in which portion of the cuticle the Tedion residue resides. As shown in table 1, approximately 90% of the Tedion residue was located within the waxy cuticle of the peel, and about 55% of the residue was coextracted with only 2 to 4% of the wax. Since the residue was not merely adhering to the surface, as shown by its resistance to detergent washing, it may be concluded that a portion

Table 2.—Residues of Tedion on and in peel of field-treated lemons before and after commercial washing operation.

Replicate	P.p.m. Tedion		Removal (%)
	Unwashed	Washed	
1	1.25	0.46	63.2
2	1.53	0.67	56.2
Average	1.39	0.57	59.0

of a persisting Tedion residue was embedded or dissolved in the outer portion of the waxy cuticle. The prolonged biological field efficiency of Tedion residues, as well as the chemical inertness and physical properties of Tedion, support this conclusion.

Since commercial washing operations in citrus packing houses result in the removal of 20 to 25% of the fruit wax (Wilson 1958), simultaneous removal of approximately one-half (see table 1) of a Tedion residue might be predicted. The concentrations of Tedion found on and in the peel of field-treated lemons before and after commercial washing, are presented in table 2, indicating that about 60% of the Tedion residue is indeed removed by the commercial washing.

References Cited

Carman, G. E., and F. A. Gunther. 1951. Factors related to the differentiation of extrasurface and subsurface residues. Presented at 35th Annual Meeting, Pacific Branch, Amer. Assoc. Econ. Ent., Seattle, Washington.

Cassil, C. C., and O. H. Fullmer. 1958. Persistence of Tedion residues on fruits. Jour. Agric. Food Chem. **6:** 908–10.

Gunther, F. A. 1958. Unpublished data.

Gunther, F. A., and R. C. Blinn. 1955. Analysis of Insecticides and Acaricides. Interscience Publishers, New York-London.

Gunther, F. A., R. C. Blinn, L. R. Jeppson, and J. H. Barkley, G. J. Frisone, and R. D. Garmus. 1957. Field persistence of the acarcidide 4,4′-dichloro-alpha-(trichloromethyl)-benzhydrol (FW-293) on and in mature lemons and oranges. Jour. Agric. Food Chem. **5:** 595–8.

Gunther, F. A., R. C. Blinn, and J. H. Barkley. 1959. Infrared procedure for the quantitative determination of residues of 2,4,4′,5-tetrachlorodiphenyl sulfone (Tedion) on and in citrus fruits. Jour. Agric. Food Chem. **7:** 104–5.

Jeppson, L. R. 1958. Unpublished data.

Wilson, C. W. 1958. Private communication.

Quadrant Distribution of Mites on Leaves of Texas Grapefruit[1]

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Abstract

Populations of three species of mites sampled from terminal-flush grapefruit leaves differed in the various quadrants from which they were collected. *Eutetranychus banksi* McG. was most abundant in the south quadrants during the year except for the 2 months following the peak population, which occurred in May 1955 and June 1956. Citrus rust mites, *Phyllocoptruta oleivora* (Ashm.), were more numerous on the east as well as the north side of the tree during 8 months of the year, and being most numerous in the northeast quadrant. In a sulfur dusted grove, *E. Banksi* mites were more numerous on the north side of the tree 1 to 2 months later than on the untreated trees, while citrus rust mites were more numerous on the east quadrants. *Brevipalpus* spp. were more common on leaves in the west quadrants.

Larger populations of the tetranychid mite *Eutetranychus banksi* McG. appeared evident on the south side of the trees during October 1954 in a study of the seasonal distribution of the various mites on grapefruit. Quadrant samples had been combined as a composite sample. Quadrant samples were segregated thereafter for a 26-month period to determine if the various mites were more numerous in certain quadrants around the tree.

Variable mite and insect populations have been reported in certain portions of fruit trees. Summers & Baker (1952) reported the possibility of drawing from heavily infested almond trees samples of leaves that give erroneous estimates of the size of the actual population of the clover mite, *Bryobia praetiosa* Koch. This mite was found to reside on the woody portion of their fruit-tree hosts, from which they made excursions to feed on leaves only during favorable periods of the day. Kremer (1956) found the clover mite to lay more winter eggs on the east and south sides of fruit trees in Germany. Friedrich (1951) reported more winter eggs of red spider mites on cherry and almond trees were laid on the side of the tree protected from the general wind direction than elsewhere. Ebeling (1950) found the average percentage of California red scale, *Aonidiella aurantii* (Maskell), distributed in the four quadrants as follows: northwest, 28.4; northeast, 43.7; southwest, 12.1; and southeast, 15.8. He reasoned the larger percentage on the north side of the tree was caused by it's being the cooler side and the sun's heat striking the tree from the south. The west side of the tree is exposed to the sun in the afternoon when the temperature is higher. Thus, he deduced, one might expect a lower population on the west side of the tree.

Methods.—Leaf samples were taken around the tree, 2 to 7 feet from the ground. The third leaf from the apical end of the terminal flush of growth was selected, varying to the second or fourth leaf as necessary. Ten leaves were selected per quadrant from each of five untreated trees and from five trees from a block treated with sulphur dust for citrus rust mite control. Segregated quadrant sampling was begun in November 1954 and extended through December 1956. Following January 1955, two additional untreated trees were sampled. A stereoscopic microscope with 18× magnification was used to count the mite populations on the leaf samples.

Results and Discussion. Certain factors were given special consideration in order that evaluation of percentages of mite populations in various quadrants might be properly interpreted. A high percentage of a very low total mite population, which may have been shown to occur in a particular quadrant, might have been due to an inadequate number of leaves in a sample or to chance expectancy when sampling mite populations. However, when a higher population consistently occurred in a particular quadrant in the succeeding month or months, a more valid high-population trend was indicated. Such questionable extremes did not show up in the 26-month averages. The terminal, mature flush of leaves invariably contained the greater population of *E. banksi*. Although rust mite populations may be very heavy on interior leaves and fruit, variations in populations of citrus rust mites, *Phyllocoptruta oleivora* (Ashm.), around the tree were explained in this investigation only in terms of those from mature terminal flush leaves. Average monthly mite populations and percentages of the population occurring in each quadrant are shown in table 1.

More time was required to count the mite population on the left side of the leaf (that area to the left of the midrib when the leaf was observed toward the apical end) both on the upper and lower surface of the leaf. Actual counts during May 1956 showed that *banksi* and rust mites were more numerous on the left side of the leaf than the right side of the leaf.

Eutetranychus banksi.—The *banksi* mite population was higher on the south side of the tree than on the north side except for the 2 months following the peak month, which had 100.9 mites per leaf in May 1955 and 59.0 in June 1956. A trend of a greater percentage of mites in the southwest sector seemed evident prior to and including the peak month with a slight shift to the southeast quadrant and then to the northwest and northeast, respectively. More than 55% of a yearly total of the mites occurred in all quadrants during May and June. The southwest and southeast quadrants had the greatest number of mites throughout the year while the northwest quadrant tended to have the smallest numbers. South-side predominance was noted particularly from October through the peak population month in the following year.

A somewhat different mite population trend followed when sulfur dust applications (for rust mite control) were applied following the monthly count in March, May, and August 1955, and February, May, and August 1956. The peak populations were delayed until the July to August period. The north side predominance prevailed during August and September 1955 and during August 1956. During this particular 26-month period, the percentage of the yearly mite total in the south quadrants

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Table 1.—Percentage of mites in various quadrants and average populations of *Eutetranychus banksi* and citrus rust mites on untreated grapefruit leaves November 1954 to December 1956.

	banksi MITES					*banksi* EGGS					CITRUS RUST MITES				
	Per Cent				Mites per	Per Cent				Eggs per	Per Cent				Mites per
MONTH	NW.	SW.	SE.	NE.	Leaf	NW.	SW.	SE.	NE.	Leaf	NW.	SW.	SE.	NE.	Leaf
January	9	32	54	5	4.5	9	39	51	1	4.5	19	25	29	27	466.8
February	5	32	42	21	1.6	6	41	43	10	1.3	23	32	17	28	163.0
March	11	39	34	16	1.8	12	42	36	10	1.7	10	36	23	31	34.9
April	17	44	31	8	1.6	20	46	23	11	1.9	29	55	6	10	19.9
May	16	31	26	27	53.0	18	35	25	22	24.4	37	22	12	29	173.3
June	16	27	32	25	44.4	21	27	26	26	36.0	41	13	17	29	422.4
July	35	26	19	20	9.4	34	21	20	25	8.3	24	12	27	37	241.1
August	25	10	29	36	5.8	28	11	32	29	5.5	21	10	25	44	88.4
September	23	30	26	21	10.9	20	32	27	21	10.9	16	19	26	39	105.2
October	23	32	29	16	11.4	13	39	37	11	8.7	22	13	18	47	254.0
November	14	44	34	8	11.1	13	46	31	10	6.6	19	24	25	32	542.6
December	7	46	37	10	8.2	8	40	44	8	5.8	12	23	37	28	364.3
Average	17	31	30	22	13.5	19	32	29	20	9.5	23	21	25	31	249.2

was approximately 33% for each of the January to June, July to August, and the September to December periods. Weather conditions varied considerably from the average during the test period.

The greatest number of *E. banksi* eggs on the untreated trees was found on the south side of the tree except during the months immediately following the peak egg populations. An early peak population during 1955 was followed by north-side predominance for a month, with rather low populations under conditions of more rain and higher relative humidity (however, much lower humidity in the March to April period than in 1956). A June peak population during 1956 was followed by north-side predominance for 2 months, with somewhat higher populations under conditions of less rain and drier conditions than in 1955. Particular south-side predominance was noted from October until the month before the peak in the following year, except during March and April 1956 (when populations were very small). South-side predominance was noted to be 95% in January 1956. The southwest quadrant showed greater numbers than any of the other quadrants before the peak and following the small spring populations.

On the sulfur-treated trees, *E. banksi* egg populations were also more numerous on the south side of the tree, with a tendency for more in the southwest quadrant and less in the northeast quadrant. North-side predominance occurred only during August (also during September 1955). South-side predominance was particularly evident from October until the peak population month.

Citrus Rust Mite.—Average percentages of citrus rust mite populations for 26 months on leaves of untreated grapefruit were distributed in the quadrants as follows: 31-NE., 25-SE., 23-NW. and 21-SW. The northeast quadrant had more rust mites more times at the various levels of population than any other qudrant. During and following the peak, *E. banski* mite populations in May and June, rust mites on leaves were more numerous on the north side of the tree and particularly so in the northeast quadrant for 5 months following the June *E. banksi* peak in 1956. An average of monthly population counts of rust mites on leaves showed 45% on the north side and 55% on the south side during the November to January period. The general monthly averages during this period showed rust mites on leaves to be more numerous on the east as well as the north side of the tree in 8 of the 12 months. The latter 6 months of the year and either January or May to June were the common periods during which this predominance occurred.

On the sulfur-treated trees, 26-month averages showed citrus rust mite populations on leaves to be distributed in the various quadrants as follows: 36-SE., 34-NE., 16-SW. and 14-NW. Only during March and April 1956, when populations were very small, rust mite populations were greater in the western quadrants. Rust mites increased in numbers somewhat every winter, but tremendous increases occurred during the 1955–1956 winter. Particular predominance in the east quadrants was found to vary from 64 to 83% of the population during this winter increase period, except during February 1956 when 25 and 16% occurred in the NW. and SW. quadrants, respectively. This latter February period followed 2 months of very heavy rust mite populations. Following a period of very small numbers, rust mite populations were very predominant in the east quadrants.

Brevipalpus spp.—General averages of false spider mite populations showed the following percentages in the various quadrants: 36-SW., 33-NW., 18-NE., and 13-SE. Larger numbers of false spider mites were found on the leaves during the July to December period when 64 to 80% occurred on the west side of the tree. A south-side predominance was noted during the lighter population months of January through March. False spider mites were very low in numbers on the leaves from April through June. No particular quadrant predominance was noted in the very small populations on the sulfur-treated trees.

Tydeid Mites.—General averages of Tydeid mites showed 23 and 27% in each of the east and west quadrants, respectively. No particular quadrant predominance was noted, although these mites were more numerous on leaves during the June to December period. Tydeid mites occurred in small numbers on the sulfur-treated trees.

Typhlodromus spp.—General monthly averages of these predaceous mites showed the following percentages in the various quadrants: 32-NW., 31-NE., 20-SE., and 17-SW.

Although these mites were more numerous from May through November, their smaller populations did not seem to show a definite pattern of increase in any quadrant.

REFERENCES CITED

Ebeling, Walter. 1950. The distribution of red scale in a citrus tree. Subtropical Entomology, p. 306–7. Lithotype Process Co., 523 Folsom Street, San Francisco, California.

Friedrick, Gerhard. 1951. Über die durch den Wind bedingte ungleiche Verteilung von Schildläusen und Wintereiern der Insekten an den Zweigen der Obstbäume. Anz. Schädlingsk. **24**: 33–4.

Kremer, Friedrich-Wilhelm. 1956. Studies on the biology, epidemiology and control of *Bryobia praetiosa* Koch. Hofchen-Briefe (English Edition) 9(4): 189–252.

Summers, Francis M., and George A. Baker. 1952. A procedure for determining relative densities of brown almond mite populations on almond trees. Hilgardia 21(13): 369–82.

The Relationship Between Insecticide-Altered Aphid Populations and the Spread of Potato Leaf Roll[1]

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ABSTRACT

Reduction of the spread of the potato leaf roll virus disease through control of the green peach aphid, *Myzus persicae* (Sulz.), on potatoes in central Washington has varied from considerable to none at all or to an increase in disease spread. In 1955 and 1956 insecticide applications made during a period when large populations of aphids were migrating to the potatoes resulted in a larger percentage of leaf-roll-induced net necrosis in the tubers than was found in the untreated plots. In addition, in 1956 treatments with Diazinon,® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), and to a lesser extent with schradan or with mixtures of the two, reduced the yields and quality of the crop. Treatments with Diazinon greatly reduced the population of wingless aphids but rendered the plants toxic to winged migrants for only a short period. Two weeks after treatment the aphid populations in such treated plots were as much as four times that on the untreated plots. The increased net necrosis and decreased yield were associated with the posttreatment increase in populations of winged and wingless aphids.

In 1957 aphid populations were small and aphid control resulted in decreased net necrosis. Weekly treatments with schradan and Diazinon during the migratory period resulted in only 7% net necrosis compared with 18% in the untreated plots. Three weekly treatments prior to July 20 resulted in 14% net necrosis while three weekly treatments after July 30 resulted in 9% net necrosis, indicating that most of the leaf roll spread came during the latter part of the aphid migratory period. Weekly applications of parathion resulted in 15% net necrosis, only slightly less than that of the check.

Reduction of the spread of potato leaf roll by means of aphid control in the Yakima Valley of Washington has varied from considerable to none at all or even to an increase in the amount of spread.

Early experiments (Gibson *et al.* 1951, Klostermeyer 1953) showed a reduction of leaf roll spread in Russet Burbank potatoes when low to moderate populations of green peach aphids, *Myzus persicae* (Sulz.) were held in check with organic phosphate insecticides. Tests in 1954 (Klostermeyer *et al.* 1956) showed no significant reduction in leaf roll spread as measured by the amount of tuber net necrosis, although the untreated plots had slightly higher net necrosis readings. Net necrosis is a tuber symptom of leaf roll resulting from current-season infection in certain potato varieties. In 1955 and 1956 additional tests of systemic and other insecticides for aphid control were conducted. The effects on the aphid populations were presented by Landis *et al.* (1958). Data on leaf roll spread in these experiments are reported herein, together with results of an additional experiment conducted in 1957.

LEAF ROLL SPREAD IN 1955.—The amount of leaf roll spread in the experiment conducted in 1955 was determined by the amount of net necrosis in the harvested tubers. Net necrosis does not affect all tubers from leaf-roll infected plants and hence does not serve as an index of absolute leaf roll spread but it is an indication of the commercial importance of the disease at harvest and is related to the amount of leaf roll spread. To provide additional information on the number of infected tubers in the harvested crop, tuber samples were planted the following spring and the number of infected plants counted. The results are in table 1.

Contrary to previous results, the percentage of net necrosis in the treated plots was in excess of that in the untreated plots. With the exception of the Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl)phosphorodithioate) and parathion treatments, there was an inverse relationship between the seasonal total aphid population and the amount of net necrosis at harvest (table 1). All treatments had more net necrosis than the check and also had lower seasonal aphid populations. Schradan, the treatment with the lowest aphid population, had the highest percentage of net necrosis.

LEAF ROLL SPREAD IN 1956.—The 1956 experiment tested schradan and Diazinon®, (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate, alone and in combination at three rates of application. The effect of aphid control on the amount of tuber net necrosis in 1956 was similar to the results in 1955; that is, the net necrosis was in inverse association with the aphid popula-

[1] Scientific Paper No. 1812. Washington Agricultural Experiment Stations, Pullman, Project 851. The work in 1955 and 1956 was in cooperation with the U. S. Department of Agriculture, Entomology Research Division, Agric. Res. Serv., Union Gap, Wash., B. J. Landis, in charge. Personnel of that laboratory made the population counts and were responsible for recording part of the leaf roll data. Their contribution is gratefully acknowledged. Accepted for publication February 16, 1959.

Table 1.—Leaf-roll infection in potatoes as measured by net necrosis and infected plants, Prosser, Wash. 1955.

Treatment and No. of Applications	Seasonal Aphid Population[a]	Percentage of Net Necrosis Tubers Examined		Percentages of Infected Plants
		October, 1955	March, 1956	June, 1956
Untreated	200,492	24.7	16.4	99.7
Guthion, 3	153,599	44.7	29.4	98.1
Chlorthion,[b] 3	151,430	26.6	18.0	98.7
Parathion, 3	83,762	58.7	27.9	99.6
Thimet,[c] 3	56,692	36.7	24.9	96.9
Dimethoate, 3	46,057	38.0	25.2	97.8
Demeton, 2	21,040	38.1	28.3	99.3
Schradan, 2	13,729	53.3	39.3	98.8

[a] Total aphids on 250 leaves in 12 samples (total of 3,000 leaves) taken at intervals from June 30 to September 1.

[b] *O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate.

[c] *O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate.

tions. In addition, the treatments which caused high aphid mortality also resulted in decreased yields, poor tuber shape, and a lower specific gravity. These data are summarized in table 2. Statistical analysis disclosed significant differences among the schradan treatment means for specific gravity but no significance among treatments for tuber shape and net necrosis. Nevertheless, the data for each factor show similar trends among treatments.

As in 1955, the 1956 crop was heavily infested by green peach aphids. Plots left untreated or treated with an insecticide of limited residual effectiveness sustained a high population of wingless aphids. Plants in these plots became sticky with honeydew and had high predator populations. Such plots seem to have repelled immigrating winged aphids. Data given in table 3 show that where Diazinon reduced the wingless aphid populations on July 16, 1956, the plots became reinfested by winged aphids on July 20 in numbers inversely associated with insecticide dosage. The resulting progeny completely reversed the previous population trend when sampled on July 26. The amounts of net necrosis in the treatments tend to be associated with these winged aphid populations of July 20 and the wingless aphid populations of July 26. There is little or no association of the high aphid populations in early July with the amount of net necrosis.

Further corroboration of these relationships is evident from the replication data. Immigrating aphids are not all from the same source and may be more abundant on one side of the field one day and on the other side the next day, depending on the source of the infestation and the direction of the wind. There was no association of aphid distribution with net necrosis by replicate in the 1955 experiment, but in 1956 the best association of aphids and net necrosis by replicate occurred on July 20, the same date that the treatment association was most pronounced. Treatments in Replicate A averaged 14.7% net necrosis and the aphid sampling totaled 3,998 aphids; Replicate B averaged 17.5% net necrosis and a total of 4,743 aphids; Replicate C had an average of 17.1% net necrosis and a

Table 2.—Yield and quality of potatoes in 1956 aphid-control experiment, Prosser, Washington.

Pounds per Acre		Seasonal Aphid Population[a]	Total Yield Tons/Acre	Specific Gravity	Per Cent		
Schradan	Diazinon				Normal Tuber Shape	Net Necrosis Oct. 1955	Leaf Roll June 1956
0	0	36,652	19.49	1.092	85	15	78
	0.5	22,126	18.47	1.091	81	26	88
	1	20,188	18.19	1.088	79	34	96
	2	17,293	16.71	1.083	53	32	94
0.5	0	10,237	20.10	1.089	76	29	87
	0.5	7,903	20.42	1.085	79	35	92
	1	11,292	18.84	1.090	76	37	89
	2	6,399	19.77	1.084	75	38	92
1	0	8,087	20.01	1.089	67	26	88
	0.5	4,017	19.77	1.087	77	27	88
	1	3,463	20.01	1.086	55	32	91
	2	4,597	20.56	1.086	67	28	92
2	0	4,410	19.26	1.087	61	27	83
	0.5	4,154	19.68	1.091	71	22	82
	1	1,999	20.94	1.091	58	28	89
	2	3,237	21.03	1.084	69	33	85
			Means of schradan treatments				
0		24,065	18.22	1.089	74	27	89
0.5		8,958	19.78	1.087	77	35	90
1		5,041	20.09	1.087	67	28	90
2		3,450	20.23	1.088	65	28	85
			Means of Diazinon treatments				
	0	14,846	19.72	1.089	72	24	84
	0.5	9,550	19.59	1.089	77	28	88
	1	9,236	19.50	1.089	67	33	91
	2	7,882	19.52	1.084	66	33	91

[a] Total aphids on 250 leaves in 7 samples (total of 1,750 leaves) taken at intervals from July 2 to August 2.

Table 3.—Aphid populations on potatoes in Diazinon treatments. Prosser, Wash. 1956.

DIAZINON[a] (LB./A.)	NO. APHIDS PER 250 LEAVES (JULY) 12	16	20	26	NET NECROSIS (%)
	Winged aphids				
0	109	45	13	29	15
0.5	98	10	21	10	26
1	106	26	162	29	34
2	262	18	144	72	32
	Wingless aphids				
0	11,725	13,777	2,927	864	
0.5	11,489	1,426	4,433	806	
1	12,062	417	2,219	2,596	
2	10,883	151	1,408	3,267	

[a] Applied June 12 and July 13. No schradan was applied.

total of 4,288 aphids. A fair degree of association of aphids and net necrosis existed among replicates on July 26 and on August 2 but none at all in earlier sampling.

The effects on yield and quality of the potatoes noted in 1956 probably resulted from the interactions of the effects of leaf-roll infection and direct aphid injury. Populations such as occurred in 1955 and 1956 tend to stunt the plants and the feeding of the large populations of aphids must cause considerable drain on the plant metabolism, but in the absence of a leaf-roll-immune potato variety it is impossible, under field conditions, to separate the effect of aphid-feeding injury from the effects of leaf-roll infection.

APHID CONTROL AND LEAF ROLL SPREAD IN 1957.—In order to determine the reason for increased amounts of net necrosis as a result of aphid control, an experiment was devised to estimate the effects of aphid control at different times within the season and with different amounts of aphid suppression.

Three insecticides at various treatment schedules were used to provide the desired aphid population conditions: schradan at 1 pound per acre per application, Diazinon at 2 pounds per acre, and parathion at $\frac{3}{4}$ pound per acre. Five treatment schedules were used with schradan: weekly applications for 6 weeks; application at 2-week intervals; two applications 21 days apart; applications weekly for 3 weeks at the first half of the season; and three weekly applications at the last half of the season. Diazinon and parathion were each applied at 1-week and at 2-week intervals. These, plus an untreated check, made 10 treatments, which were arranged in a randomized block design of 4 replications.

Treatments were made with a tractor-mounted sprayer having four hollow cone nozzles per row, mounted on trailing skids with two spraying downward and outward and two spraying upward and outward. Vine spreaders ahead of the nozzle lifted the lower leaves of the plants, permitting thorough spray coverage. Applications were made at 20 gallons of dilute spray per acre at 60 pounds pressure. All insecticides were used as emulsifible concentrates. Aphid populations were determined by picking 25 lower leaves from each plot. The aphids were removed with a mechanical brushing machine and deposited on a rotating sticky plate. The aphids within two cross strips of the plate involving one-fifth of the plate area were counted. The first spray application was made July 9, shortly after the migrating aphids began to infest the plants. The final application was made August 13. The last aphid sample was taken August 23 when the aphid population was very low and the foliage maturing. The potatoes were harvested October 15.

Table 4.—Wingless aphid populations on potatoes, Prosser, Wash. 1957.

TREATMENT[a]	WINGLESS APHIDS PER 100 LEAVES July 12	July 19	July 26	Aug. 2	Aug. 9	Aug. 16	Aug. 23	SEASONAL TOTAL
Schradan								
1. 7 days	5	10	15	30	0	0	0	62
2. 14 days	35	190	25	55	4	8	20	337
3. 21 days	0	80	90	20	5	22	17	234
4. 1st half	0	5	5	30	18	30	23	111
5. 2nd half	140	440	215	75	3	2	2	877
Diazinon								
6. 7 days	5	25	30	10	1	1	13	85
7. 14 days	10	375	45	255	1	43	46	775
Parathion								
8. 7 days	25	45	75	30	11	19	79	284
9. 14 days	35	350	15	130	53	105	89	777
10. Untreated	165	350	135	170	137	66	20	1043

[a] Treatments No. 1, 6, and 8 were sprayed on July 9, 16, 23, 30, Aug. 6 and 13.
Treatments No. 2, 7, and 9 were sprayed on July 9, 23 and Aug. 16.
Treatment No. 3 was sprayed July 9 and July 30.
Treatment No. 4 was sprayed July 9, 16, and 23.
Treatment No. 5 was sprayed July 30, Aug. 6 and 13.

Results of the aphid sampling are given in table 4. The data on yields and net necrosis and leaf-roll infection are shown in table 5. Differences among the seasonal average aphid populations were significant at the 1% level. Differences in yield were not significant but differences in the percentage of net necrosis were significant at the 5% level. No significant differences were found among specific gravity readings or in tuber shape.

The seasonal distribution of the aphids in 1957 indicated that much of the leaf roll spread occurred in the last half of the season, just as it did in 1956. Thus, treatment 5 (tables 4 and 5) had 9% net necrosis under conditions of high early-season and low late-season populations. Treatment 4 had 14% net necrosis with aphids suppressed during the first part of the season and allowed to increase during the latter part of the season. The same relationship held for Treatments 6 and 7. Weekly applications of Diazinon held the late-season population in check but

Table 5.—Yield of potatoes and leaf roll infection, Prosser, Wash. 1957.

TREATMENT	YIELD TONS/ ACRE	PER CENT LEAF ROLL INFECTED PLANTS (AUG.) 6	20	28	TUBER NET NECROSIS (%)
Schradan					
1. 7 days	11.75	1.8	3.5	6.6	7.3
2. 14 days	12.21	1.9	3.3	4.6	7.0
3. 21 days	13.64	2.4	4.9	8.1	12.8
4. 1st half	12.93	1.4	4.3	6.9	14.3
5. 2nd half	12.21	2.9	4.5	5.6	8.8
Diazinon					
6. 7 days	11.78	2.5	4.6	5.4	7.8
7. 14 days	12.64	2.3	6.1	6.1	11.3
Parathion					
8. 7 days	13.67	2.6	5.9	6.9	15.0
9. 14 days	13.76	1.9	6.4	8.6	14.3
10. Untreated	12.67	3.3	6.4	8.3	18.3

every-other-week applications did not. With the parathion Treatments 8 and 9, weekly applications evidently did not suppress the populations enough to reduce the amount of net necrosis. The plants were examined for leaf-roll symptoms periodically in August. The greatest increase in infected plants occurred between August 20 and August 28 (table 5). The best association of aphid populations with net necrosis was on August 16 for both the replicate data and the treatment data.

DISCUSSION.—Since green peach aphids are present on potatoes during most of the growing season, leaf-roll transmission can occur at any time and plants may be infected repeatedly. Aphid populations can be sampled only at intervals and there is no practical way of determining the percentage of the aphid population which is viruliferous. Because of this and because the aphid population changes daily during the season, it is difficult to find a clear-cut correlation between aphid populations and leaf roll spread.

In all of these experiments, certified seed containing less than 1% of leaf roll was planted. It is likely that the initial leaf roll spread in the crop resulted from infectious aphids that acquired the virus from the early crop potatoes on which they developed. Immigrating winged aphids move from plant to plant, depositing young and feeding, and in the process can infect many plants. Wingless aphids move less freely and usually only to adjacent plants.

Where aphid migration is low and of short duration large populations of wingless aphids do not develop and the plants remain attractive to winged migrants regardless of whether they have been treated with insecticides. Leaf roll spread is at a minimum and aphid control results in reduced leaf roll spread.

When the crop is subject to high populations of winged migrants for 3 or 4 weeks, the plants soon become heavily infested with wingless aphids and apparently no longer attract large numbers of migrants. Leaf roll spread may then be due mostly to movement of wingless aphids from over-crowded infected plants to adjacent plants. Insecticide treatment keeps the wingless aphid population at a low level and the plants remain attractive to winged migrants. These more mobile forms, which may be already viruliferous from having fed on diseased plants in the early potato fields in which they developed, may spread the disease widely and the net result will be an increase in leaf-roll disease spread even though the total aphid population on the treated plants may be low.

Control of the spread of the leaf-roll virus disease by means of aphid control seems practical only under conditions of low-aphid infestation (less than 10 aphids per leaf). Under high-aphid infestations, insecticide application may not reduce leaf roll spread but it might be necessary to prevent direct damage to the plants from aphid feeding. In either case, control measures once begun must be continued until the aphid immigration has abated.

REFERENCES CITED

Gibson, K. E., B. J. Landis, and E. C. Klostermeyer. 1951. Effect of aphid control on the spread of leaf roll in potatoes. Amer. Potato Jour. 28:7;: 658–66.

Klostermeyer, E. C. 1953. Entomological aspects of the potato leaf roll problem in central Washington. Washington Agric. Expt. Sta. Tech. Bull. 9.

Klostermeyer, E. C., B. J. Landis, Ralph Schopp, and Lillian I. Butler. 1956. Effect of systemic insecticides on green peach aphid populations on potatoes. Jour. Econ. Ent. 49(2): 164–6.

Landis, B. J., Ralph Schopp, and E. C. Klostermeyer. 1958. Further studies with systemic insecticides against the green peach aphid on potatoes. Jour. Econ. Ent. 51(2): 138–40.

Biology and Control of *Centrinaspis capillatus* (Lec.) in Redtop[1]

WILLIAM H. LUCKMANN and CHARLES D. LESAR[2]

ABSTRACT

A small weevil, *Centrinaspis capillatus* (Le Conte), is a serious pest of redtop in Illinois, and it frequently reduces seed production by as much as 75%. The weevil overwinters in the adult stage in the soil in fields of redtop. It leaves hibernation in May and soon begins to oviposit in stems of the host plant. The egg stage lasts 8 to 10 days. The larva feeds for 30 to 40 days in an internode of the stem and then crawls into the soil where it remains relatively inactive for another 30 to 40 days before pupating. The pupal stage lasts 10 to 14 days. There is one generation each year. *Centrinaspis capillatus* (Le Conte) is present in redtop throughout southern Illinois but it is not recorded from northern Illinois. Tests with insecticides indicate ¼ pound of dieldrin or ½ pound of heptachlor per acre will control this insect.

Redtop, *Agrostis alba* L., is a perennial grass of European origin. According to Burlison (1934), it was first grown in Illinois about 1867. At the present time, 95% of all redtop seed produced in the United States and 85% of the world production of seed originates in 12 counties of southern Illinois (1957 Illinois Annual Farm Census).

In southern Illinois, a small weevil, *Centrinaspis capillatus* (Le Conte) is a serious pest of redtop and it frequently destroys up to 75% of the seed crop. Le Conte described the adult weevil in 1876 and Casey (1920) gives a good description of the genus. However, as this insect has no common name approved by the Entomological Society of America, for the purpose of convenience it will be referred to as the "redtop weevil."

BIOLOGY.—The study of the life history of the redtop weevil was begun in 1956 with some preliminary observations in Fayette County, Illinois. Research was continued in a more detailed manner in 1957 and 1958 in Clay and Marion Counties in southern Illinois. The data obtained indicated that the redtop weevil has only one generation each year.

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The small grayish-black weevil overwinters in the soil as an adult. Individuals begin to leave hibernation in early May, and by June all weevils have left the soil. Adults are present until mid-July when they disappear and presumably die. Thus, in southern Illinois adults are present in fields of redtop from about May 5 to July 15.

After the adults leave the soil, they feed on the tender, newly expanded leaves of redtop and, later, on the panicle or seed stalk. Adults mate soon after leaving hibernation and egg laying begins within 7 to 10 days. Heaviest egg deposition occurs during the first part of June and appears to coincide with peak numbers of adults. Eggs are present, however, from mid-May to late June.

The gravid female chews a hole approximately 1 mm. in diameter, and, reaching in with the tip of the abdomen, deposits a single, cream-colored egg in the center of the hollow internode of the stem. The egg may adhere to the spot where it is deposited or it may roll to the bottom of the hollow internode. The position of the oviposition scar, the frass, and the position of the larva indicate that many of the eggs may roll down to the base of the hollow internode before hatching. They hatch in 8 to 10 days. Only in rare instances are two eggs laid in the same internode. When two eggs or two larvae are present in the same internode there are also two oviposition scars.

The yellowish-white larva feeds 30 to 40 days on the inside of the internode. It does not cut through a node and feed into adjacent internodes. The first larval stadium lasts 8 to 10 days. The second stadium lasts 12 to 14 days. The first two stadia and a part of the third are completed in the stem.

Normally only one larva is found in an internode but individual stems may contain two or more larvae. The first and second internodes below the seed stalk are the most preferred locations for egg deposition and about 80% of the eggs or larvae are in these two internodes.

The third instar larva feeds in the stem and then cuts an exit hole at the top of the internode and drops to the soil. The abandoned internode is packed with frass and may be partly severed at the base. After dropping to the ground the larva burrows to a depth of 1 to 5 inches, depending on conditions in the soil. Individuals tend to burrow deeper in drier soils. Once in the soil the larva prepares a chamber or earthen cell and becomes relatively inactive for 30 to 40 days. Shortly before pupating, the larva relocates in the upper 3 inches of soil and again constructs an earthen cell. The pupal stage lasts 10 to 14 days. Following emergence the adult remains relatively inactive in the soil until the following spring. The life history of the redtop weevil is illustrated in figure 1.

ECOLOGY.—The redtop weevil is found throughout southern Illinois wherever redtop is grown. It is found in redtop along roadsides, railroad rights of way, in mixed pastures, and in fields that are used for producing redtop seed. The weevil is apparently restricted to redtop and it tends to migrate readily if ecological conditions are not suitable. Cutting of redtop for hay, for example, causes considerable migration into neighboring fields. Occasionally an adult specimen is collected on other grasses and alfalfa, but if redtop plants are near, the presence of the weevil on other plants is in all probability only temporary.

The redtop weevil has never been recorded from northern Illinois and it was not found in that area in 1957 or 1958, even though redtop is fairly abundant in some counties. Whether the redtop weevil eventually kills a plant or merely causes injury to it has not been determined. Evidence indicates that injury to the stem and seed stalk is not directly responsible for reduction and possible elimination of the plants. Scattered clumps of redtop are fairly common in pastures and abandoned fields in southern Illinois, and these clumps are usually heavily infested with weevils. Although an infested plant is capable of sending out new leaves from nodes lower on the stem, competition with other plants in the production of new seedlings and reduction of vigor could result in a gradual reduction or elimination of redtop in a field.

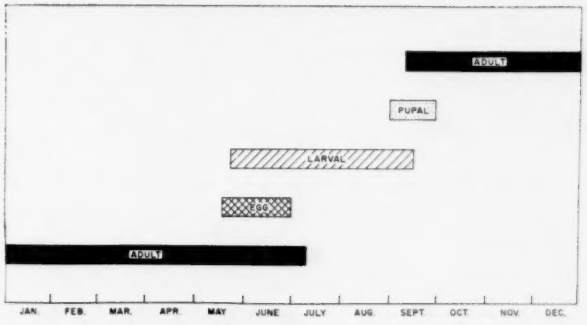

FIG. 1.—Seasonal occurrence of the stages of *Centrinaspis capillatus* (LeC.) in southern Illinois.

The seed head of an infested plant will produce little or no seed. The amount of seed produced depends on how early the stem is infested. Plants infested before the head emerges will produce no seed and, shortly after its emergence from the sheath, the head begins to dry. Plants infested later in the season or after the heads emerge may appear normal until near harvest, when the head dries prematurely. Some light seed (seed which did not fully develop) may be produced on these heads.

Redtop seed is harvested by (1) mowing and threshing, and (2) by combining. Mowing is usually done before the larvae complete development in the stem, while the combining operation takes place after most of the larvae have entered the soil. The practice of combine harvesting is increasing in Illinois and this method favors survival of the weevil.

CONTROL.—Preliminary observation in 1956 indicated that in order to acquire good experimental results with insecticides, the experimental area must be large enough to compensate for normal habits of the weevil. In 1956 it was observed that the weevils are capable of moving extensively and cannot successfully be kept out of small treated areas. Further observations indicated that insecticides applied on June 10, 1956, did not give satisfactory control of the weevil. Thus, in 1957 and 1958, insecticides were applied to individual fields or to sizable areas within a large field. Application was made soon after the beginning of egg deposition. To ascertain when the first eggs were being deposited, female weevils were dissected and examined with a microscope. Gravid females were observed on May 16 in both 1957 and 1958. Treatment with insecticides was made on May 22 to 24, 1957, and on June 1 to 2, 1958. The treatments were made with

Table 1.—The effectiveness of three insecticides applied in test I to fields of redtop for control of *Centrinaspis capillatus* in 1957.

Insecticide	Dosage (Lb./A.)	Number of Weevils per Sweep (A) and Per Cent Reduction of Weevils (B)									Per Cent		Seed Yield (Lb./A.)
		May 22	May 31		June 6		June 13		June 19		Infested Stems	Reduction on July 7	
		A	A	B	A	B	A	B	A	B			
Dieldrin	0.25		1.1	79.3	1.6	72.9	0.7	78.2	0.3	82.4	12	84.5	225
Heptachlor	.50		0.8	85.0	0.7	88.2	0.4	87.5	0.5	70.6	3	96.1	—
Methoxychlor	2.00		2.2	58.5	3.2	45.8	2.4	25.0	1.7	—	72	6.5	—
None		1.3	5.3	—	5.9	—	3.2	—	1.7	—	77	—	78

a tractor-mounted sprayer applying 15 gallons of finished spray per acre.

Three insecticides were used in 1957 at the following dosages: ¼ pound of dieldrin per acre, ½ pound of heptachlor per acre, and 2 pounds of methoxychlor per acre. Dieldrin and heptachlor gave excellent control of the weevil and reduced the percentage of infested stems 84.5% and 96.1%, respectively. Methoxychlor did not control the weevils.

Results of the insecticide tests in 1957, given in table 1, do not express dramatically the visible difference between the treated and untreated redtop. Although these data show only 77% of the stems in untreated fields were infested, such an infestation makes it unprofitable to harvest the remaining crop.

Dieldrin and heptachlor were tested again in 1958, and DDT at 1 pound per acre was substituted for methoxychlor. As the data in table 2 illustrate, dieldrin and heptachlor gave good control in 1958, but DDT was less effective in reducing the number of weevils. Thus, it was anticipated that the yield of seed in the DDT-treated plots would be considerably less than the yield from plots treated with the other two materials. The increase in yield in the DDT plots, however, was greater than the increases in yield in the plots treated with dieldrin or heptachlor. Experimental results are frequently difficult to explain, but it is possible that DDT controlled more of the other insects present, of which leafhoppers were the most abundant.

To evaluate further the losses caused by the weevil, the highest number of adults collected in each plot on any date during June, the percentage of stems infested, and the percentage of reduction in yield of seed are graphically illustrated in figure 2. Data for these calculations are presented in tables 1 and 2, although the percentage of stems infested, as shown in figure 2, is based on the difference between the number of infested stems in the treated and untreated plots. These calculations show that treatment with an insecticide is probably justified at populations of less than one weevil per sweep of a 15-inch net. One weevil per sweep will cause approximately 20% reduction in yield of seed.

Conclusion.—*Centrinaspis capillatus* (Le Conte) is a serious pest in southern Illinois where redtop is grown commercially for seed. During the 2 years of detailed study of the biology and control of the weevil it was established that:

1. *Centrinaspis capillatus* (Le Conte) has only one generation a year in Illinois, which is completed about September 1. Overwintering adults leave hibernation in May to lay eggs for the first generation.

2. There are three larval instars. The first two instars are completed in the stem of the redtop plant. The third instar is completed partly in the redtop stem and partly in the soil at the base of the plant. The first instar lasts 8 to 10 days, the second lasts 12 to 14 days, and the third lasts 30 to 40 days.

3. The weevil pupates in the soil. Ten to 14 days are

Table 2.—The effectiveness of dieldrin, heptachlor, and DDT applied in a commercial manner to fields of redtop for control of *Centrinaspis capillatus* in 1958.

Insecticide	Dosage (Lb./A.)	Number of Weevils per Sweep (A) and Per Cent Reduction of Weevils (B)							Per Cent		Seed Yield (Lb./A.)
		May 30	June 9		June 17		July 1		Infested Stems	Reduction on July 9	
		A	A	B	A	B	A	B			
Test I											
Dieldrin	0.25		0.08	94.4	0.08	94.7	.12	85.7	9	79.1	289
Heptachlor	0.50		.08	94.4	.08	94.7	.08	91.5	5	88.4	301
DDT	1.00		.44	68.0	.48	66.0	.50	76.2	17	60.5	307
None		1.1	1.36	—	1.50	—	.84	—	43	—	277
Test II											
Dieldrin	0.25		0.04	95.5	0.48	61.3	.04	85.7	13	77.2	101
Heptachlor	0.50		.08	90.1	.44	64.5	.04	85.7	12	79.0	101
None	—	0.75	.88	—	1.24	—	.28	—	57	—	79
Test III											
Dieldrin	0.25		0.04	96.8	2.01	19.3	.18	82.2	14	77.1	134
Heptachlor	0.50		.06	95.2	1.96	26.2	.28	74.7	11	82.0	159
None		1.0	1.32	—	2.52	—	1.35	—	61	—	111

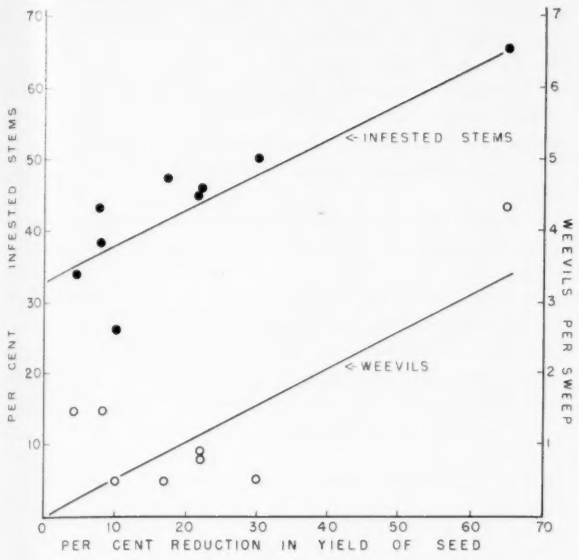

FIG. 2.—Reduction in yield of redtop seed caused by *Centrinaspis capillatus* (LeC.)

required to complete the pupal period. With the exception of the active mating, egg-laying and feeding period in May, June, and early July, the adult stage is spent in the soil.

4. Both the adult and the larva damage redtop. Relatively low numbers of weevils are capable of doing enough damage to warrant treating with insecticide. A single application of insecticide, properly timed, will give good control of the weevil. In Illinois, treatment at the beginning of egg laying, during the last 10 days of May, provided the best control. Dieldrin at $\frac{1}{4}$ pound or heptachlor at $\frac{1}{2}$ pound per acre gives very good control of the weevil. DDT appears to give best control of the entire insect complex found in redtop.

5. Although redtop is grown throughout Illinois, *Centrinaspis capillatus* is found only in the southern third of the state.

REFERENCES CITED

Burlison, W. L. 1934. Production and marketing of redtop. Univ. Illinois Agric. Expt. Sta. Bull. **404**: 231–99.

Casey, T. L. 1920. Some descriptive studies among the American Barinae. Mem. Coleoptera 9(3): 393–408.

Illinois Annual Farm Census. 1957. Released by Illinois Co-Operative Crop Reporting Service.

Le Conte, J. L., and G. Horn. 1876. The Rynchophora of America north of Mexico. Proc. Amer. Phil. Soc. 15(96): 309.

Cross Resistance in a Diazinon-Resistant Strain of *Musca domestica* (L.)[1]

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ABSTRACT

Laboratory selection of a Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl phosphorothioate)-resistant field strain of house fly, *Musca domestica* (L.), for nine generations on Diazinon-treated panels increased resistance not only to Diazinon but to a wide variety of insecticides. The levels of resistance to the various materials, as determined by topical application, were: Diazinon (38-fold), parathion (16), ronnel (18), Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate) (23), malathion (5), Sevin® (1-naphthyl-*N*-methyl carbamate) (more than 37-fold), Isolan® (dimethyl 5-(1-isopropyl-3-methyl-pyrazolyl) carbamate) (14), Pyrolan® (dimethyl 5-(3-methyl-1-phenylpyrazolyl) carbamate) (10), chlordane (400), dieldrin (220), lindane (250), DDT (more than 6,400-fold), TDE (more than 1,300), methoxychlor (more than 3,600), Dilan® (1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts (1,1-bis(*p*-chlorophenyl)-2-nitrobutane) (10) allethrins (5), pyrethrins (4), and Lethane 384® (2-(2-butoxyethoxy)ethyl thiocyanate) (2-fold). These increases in tolerance, except to Lethane, pyrethrins, allethrins, and malathion (which may be simply instances of vigor tolerance), are considered true resistances. The extent of cross-resistance indicates that most of the contemporary materials will be ineffective against Diazinon-resistant flies. It is suggested that phosphorodithioates, more stable carbamates, or more toxic thiocyanates may offer promise for control.

Tests on resistant flies collected from the field without further selection on Diazinon in the laboratory indicate that the pattern of resistance determined in this study is very similar to that which occurs under completely natural conditions of selection.

Resistance to Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) in field strains of house fly, *Musca domestica* (L.), was first reported in Denmark in 1955 (Kieding 1956). Similar occurrences were observed in the United States in 1957 by Hansens (1958) in New Jersey, by Schoof & Kilpatrick (1958) in Georgia and Arizona, and by LaBrecque *et al.* (1958) in Florida. In laboratory tests with treated panels, Hansens (1958) found that the most resistant of several strains from various dairy barns in New Jersey was almost completely unaffected by 5 minutes' exposure to 1.0 mg. of Diazinon per square inch. This concentration gave 100% mortality with a laboratory-susceptible strain (Wilson line) of house fly. After three laboratory selections with Diazinon the resistance of the field strain had increased

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to the point where 99% survived 15 minutes' exposure to 1.0 mg. per square inch. Similar panel tests with DDT, methoxychlor, chlordane, lindane, malathion, parathion, dicapthon, ronnel, Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), and Co-ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) indicated a marked resistance to these materials as well.

The dairy barn from which the strain was collected had received a spray of 1% Pyrolan® (dimethyl 5-(3-methyl-1-phenylpyrazolyl) carbamate) plus 1% methoxychlor in August 1952, and from then until October 1957, when collections of the strains were removed to the laboratory, Diazinon was the sole treatment; during that period a total of nine sprays of 1% Diazinon had been applied.

Resistance to Diazinon poses a serious problem in fly control because of the accompanying cross-resistance to other insecticides. The present study was undertaken to determine the extent of this cross-resistance, in the belief that the information will prove useful in finding chemicals suitable for control of Diazinon-resistant insects.

EXPERIMENTAL RESEARCH.—All insecticide solutions were prepared with A.C.S. grade acetone immediately before use. The following compounds were tested: pyrethrins (20%), allethrins (94.8%), Diazinon (94.5%) ronnel (purified), Isolan® (dimethyl 5-(1-isopropyl-3-methylpyrazolyl) carbamate) (purified), Pyrolan (purified) Lethane 384® (2-(2-butoxyethoxy)ethyl thiocyanate) (95%), pp-DDT (purified), TDE (purified,) methoxychlor (purified), lindane (99+%), dieldrin (99.9%), parathion (98.5%), malathion (95%), Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate) (purified), chlordane (technical), Dilan® (1 part 1,1-bis(*p*-chlorophenyl)-2-nitropropane and 2 parts (1,1-bis(*p*-chlorophenyl)-2-nitrobutane) (purified), and Sevin® (1-naphthyl-*N*-methyl carbamate) (technical).

The Diazinon-resistant house fly strain (field-collected) was subjected further to Diazinon pressure in the laboratory for nine generations, starting with 1.0 mg./sq.in. for 15 minutes (5 generations) and terminating with 10 mg./sq. in. for 15 minutes (4 generations). One-day-old flies were used in all selections.

The degree of resistance was determined by comparison toxicity tests on the selected strain and a susceptible laboratory line (Wilson) which was reared under conditions closely approximating those used for the resistant strain.

Dosage mortality data were obtained by topical application to 4-day-old unsexed flies. It is recognized that the females as a rule are naturally more tolerant and that the slope of the log dosage-probit (ld-p) line is not necessarily the same for the two sexes. However, it was reasoned that the natural differences between sexes were small compared with the magnitude of resistance indicated by panel tests, and, consequently, the text individuals were not sexed.

For treatment the flies were anesthetized with CO_2 and placed in petri dishes. Pieces of dental wick saturated with skim milk were provided as a source of food. Acetone solutions of insecticides were applied to the thorax in 0.2 ± 0.02 µl. quantities using a foot-operated, piston-driven, calibrated assembly. Each dish contained 10 flies and a total of 30 flies was used for each concentration of insecticide. Mortality readings were taken 24 hours after treatment, all flies incapable of locomotion being considered as dead. Both strains of flies were treated on the same day and replicate tests were conducted on different days. Each material was evaluated at five or more concentrations and each test was repeated at least twice. Dosages were calculated using the average of fly weights over the course of the experiment (Wilson strain 16.7 ± 1.7 mg.; Resistant strain 17.9 ± 1.8 mg.).

Log dosage-probit (ld-p) lines were fitted to the data by the method of least squares. The LD50s, LD95s and regression coefficients were calculated.

RESULTS.—The topical LD50s and LD95s for 18 insecticidal materials are shown in table 1. These include five types of insecticides: plant derivatives, thiocyanates, carbamates, organophosphorus compounds, and chlori-

Table 1.—Toxicity of various insecticides to a Diazinon-selected (field and laboratory) resistant field strain and a susceptible strain (Wilson) of house fly.

	Susceptible Strain		Resistant Strain			
Insecticide	LD50 (µg./gm.)	LD95 (µg./gm.)	LD50 (µg./gm.)	Increase in LD50	LD95 (µg./gm.)	Increase in LD95
Diazinon	2.52	6.60	95.3	38×	280	42×
Allethrin	24.6	138	129	5×	436	3×
Pyrethrins	24.0	96.0	84.0	4×	241	3×
Lethane-384	390	816	785	2×	2,010	2×
Malathion	21.6	57.6	112	5×	336	6×
Parathion	0.901	2.22	14.5	16×	48.0	22×
Ronnel	2.34	5.82	43.6	18×	101	17×
Chlorthion	11.4	72.0	268	23×	875	12×
Sevin	162	3840	> 6,000	> 37×	—	—
Isolan	17.4	186	252	14×	895	5×
Pyrolan	73.8	528	767	10×	6,150	12×
DDT	7.50	26.4	>48,000	>6,400×	—	—
TDE	37.2	93.0	>48,000	>1,290×	—	—
Methoxychlor	13.2	66.2	>48,000	>3,600×	—	—
Chlordane	3.12	18.0	1,232	396×	672,000[a]	37,000×
Dieldrin	0.384	1.74	84.1	220×	3,580[a]	2,060×
Lindane	0.552	1.98	134	254×	14,000[a]	7,050×
Dilan	5.88	19.2	56.0	10×	145	8×

[a] These values were obtained by extrapolation of the ld-p lines. The highest mortalities obtained were 80% for lindane, 70% for chlordane, and 70% for dieldrin

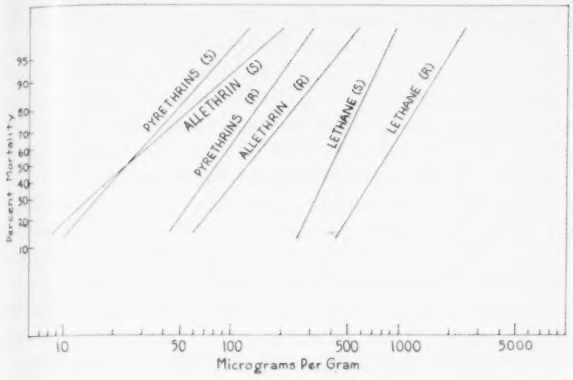

FIG. 1—Log dosage-probit curves for a Diazinon-resistant strain and a susceptible strain of house fly to allethrins, pyrethrins, and Lethane 384.

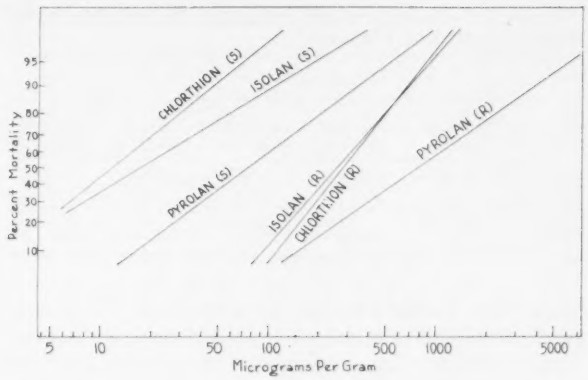

FIG. 3—Log-dosage-probit curves for a Diazinon-resistant strain and susceptible strain of house fly to Chlorthion, Pyrolan, and Isolan.

nated hydrocarbons. The dosage-mortality lines are presented in figures 1 to 5. Curves are not shown for DDT, methoxychlor, TDE, and Sevin in the case of the resistant strain, since the resistance to these materials was so high that it was impossible to obtain sufficient dosage-mortality data.

The resistant strain showed increased tolerance to all test materials, although the magnitude varied considerably from one group of compounds to another. The highest degree of resistance was found to the chlorinated hydrocarbons, particularly DDT, TDE, and methoxychlor, which were ineffective even when applied as a 40% solution. Resistance to the chlorinated cycloalkanes (chlordane, dieldrin, and lindane), although less than that to the DDT-type materials, was quite pronounced, the LD50s being 200- to 400-fold greater than for the susceptible strain. Resistance to Diazinon was next in order, there being a 38-fold increase, followed by Chlorthion (23×), parathion (16×), and ronnel (18×). It is interesting that malathion tolerance had increased only 5-fold. The increases in resistance to Isolan, Pyrolan, Dilan, allethrins, and pyrethrins were 14-, 10-, 10-, 5- and 4-fold, respectively. Tolerance to Sevin had also risen very appreciably, no mortality being obtained at a dosage 37-fold greater than the LD50 for susceptible flies. At that point the limit of solubility in acetone had been reached, thus prohibiting further testing.

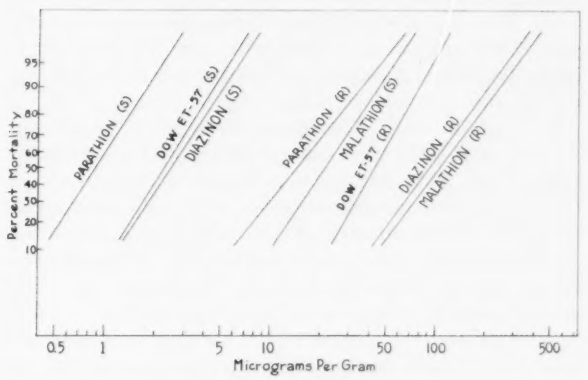

FIG. 4—Log dosage-probit curves for a Diazinon-resistant strain and a susceptible strain of house fly to parathion, Dow ET-57 (ronnel), malathion, and Diazinon.

The smallest increase in tolerance was found to Lethane 384, which was about one-half as toxic to the resistant strain.

Ten months after the resistant strain had been col-

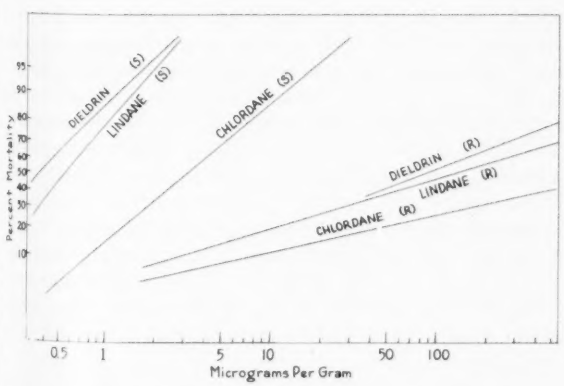

FIG. 2—Log dosage-probit curves for a Diazinon-resistant strain and a susceptible strain of house fly to dieldrin, lindane, and chlordane.

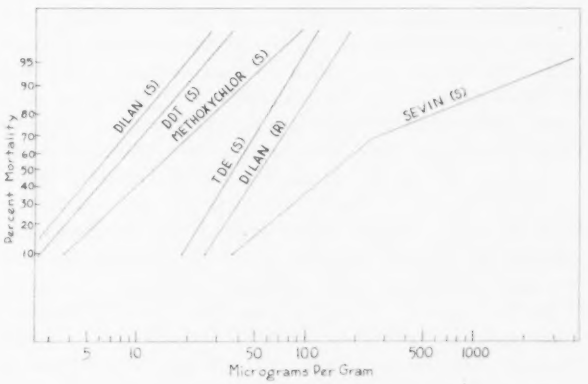

FIG. 5—Log dosage-probit curves for a Diazinon-resistant strain and a susceptible strain of house fly to Dilan, DDT, TDE, Sevin and methoxychlor.

Table 2.—Comparison of resistance in two house fly strains selected on Diazinon.

Compound	Laboratory Susceptible Strain LD50 (μg./Fly)	Field and Laboratory Selected Strain LD50 (μg./Fly)	Field and Laboratory Selected Strain Degree of Resistance	Field-Selected Strain LD50 (μg./Fly)	Field-Selected Strain Degree of Resistance
Diazinon	0.042	1.7	40-fold	0.88	20-fold
Malathion	0.36	2.0	6	1.8	5
Pyrethrins	0.40	1.5	4	1.6	4
Dieldrin	0.0064	1.5	240	0.47	73
DDT	0.13	>800	>6,000	>20	>150

lected from the field for further selection in the laboratory, additional collections were made in another barn in which resistance had been observed also the previous year. At the time of the original sampling the level of resistance in the second barn, as measured by panel tests, was slightly less than that of the population from which subsequent laboratory selections were made. During the summer of 1958 this second barn had been sprayed with 1.0% Diazinon and 1.0% malathion plus sugar and for part of the period with pyrethrum aerosols from automatic dispensors releasing small dosages at 15-minute intervals.

Drop tests with Diazinion, malathion, pyrethrins, dieldrin, and DDT were made on the collections from the second barn in the absence of laboratory selection, and the results were compared with data from similar tests on the original resistant field strain which had been further selected under laboratory conditions. Although the magnitude of resistance was less in the straight field strain (table 2), the cross-resistance pattern was similar to that found in the strain which had been collected the previous year and further selected in the laboratory.

Discussion.—Multiresistance to insecticides as the result of selection with a single material has been recognized for some time. As a rule, resistance to one chlorinated hydrocarbon confers some degree of resistance to other chlorinated hydrocarbons, but none, or at the most very little, resistance to organophosphorus compounds (Brown 1958). For example, one particular house fly strain having an extreme resistance to DDT, as well as a high degree of resistance to other chlorinated materials, showed but a 7-fold increase in KD50 to parathion (Pimental *et al.* 1953). On the other hand, selection with one organophosphorus compound not only begets resistance to related materials, but also an extremely high cross-resistance to chlorinated hydrocarbons. This was typified in the studies of March *et al.* (1956), who demonstrated that house fly resistance to DDT increased 3,000- and 600-fold by selection with parathion and Diazinon, respectively. Cross-resistance to chlorinated materials by selection with pyrethrins has been observed also (Decker & Bruce 1952, Harrison 1950), as well as cross-resistance in the reverse direction, although in the latter instance the tolerance was sufficiently low that it is open to argument whether this was resistance in the accepted sense of the word.

In the present study it was found that Diazinon-resistance was accompanied by various degrees of tolerance to all other materials tested (table 1). It cannot be assumed in all cases that the increases in tolerance were due exclusively to Diazinon pressure, for there are no quantitative data on the susceptibility of this particular population to the various materials prior to the introduction of Diazinon. Actually, in 1951 there was resistance to methoxychlor in this same barn as evidenced by control failure (Hansens 1958), and it is probable that the population was also resistant to DDT, TDE, and those other materials to which there is usually cross-tolerance. The status of cycloalkane susceptibility in the barn in question prior to Diazinon treatment is not known; however, since in the surrounding area resistance to chlordane, dieldrin, and lindane was prevalent (Hansens 1953), it is possible that some degree of resistance was already present in the population, despite the fact that cycloalkanes had never been used in this barn. Although the initial level of resistance to these materials is unknown, there is no doubt that it was increased substantially by selection with Diazinon (Hansens 1958). If LD95s, rather than LD50s, are used the differences become considerably greater. This method, however, seems a much less valid measure of resistance, if only for the reason that in some cases it is impossible to apply the amount of toxicant theoretically needed for 95% kill. This fact is not intended to imply that comparison of LD50s is an entirely satisfactory means of measuring resistance, for at high dosage rates it would seem that the proportion of applied dose available to the insect decreases with increasing concentration.

Resistance to DDT, TDE, and methoxychlor was even higher than that to the chlorinated cycloalkanes, the flies being able to tolerate applications of 40% solutions (48,000 μg./gm.). At dosages of 6,000 to 48,000 μg./gm., the mortality fluctuated between 3% and 21% and appeared to be independent of concentration. This result may have been a consequence of heterogeneity in the strain or simply a manifestation of physical action such as blocking of the spiracles by crystallization of the insecticide. For convenience in comparing with other materials, it may be stated that the increase in resistance to DDT, methoxychlor, and TDE was greater than 6,400-, 3,600-, and 1,300-fold, respectively.

This high cross-resistance to chlorinated hydrocarbons agrees with previous studies on Diazinon-resistance in laboratory strains (March *et al.* 1956; Weismann 1956, Ascher 1958). There is, however, one notable exception which illustrates that Diazinon resistance is by no means stereotyped. Working with a laboratory-selected strain of Diazinon-resistant flies, Meltzer (1956) found the usual high degree of cross-resistance to chlorinated cycloalkanes, but, oddly enough, the increase in resistance to DDT was not much greater than that for Diazinon.

There appear to be two major types of resistance to chlorinated hydrocarbons: one to DDT and its analogues, and the other to lindane, chlordane, heptachlor, aldrin, dieldrin, and toxaphene as a group (Brown 1958). Although the mechanism of DDT-resistance is still a moot question, evidence is overwhelming that DDT-dehydrochlorinase plays a major role. The resistant strain involved in the present study possesses a high concentration of DDT dehydrochlorinase (Forgash, unpublished data) which may account for its resistance to DDT-type materials.

Very little is known about the metabolism of chlorinated cycloalkanes, but the similarity of the compounds and the fact that resistance to any member of the group confers cross-resistance to the others suggests detoxifica-

tion by an enzyme system with broad substrate requirements (Hoskins & Gordon 1956). Thus, at this point it appears that the pathway to Diazinon-resistance involves selection for at least two other detoxifying systems, one for DDT-type materials and the other for chlorinated cycloalkanes.

The occurrence of cross-resistance to parathion and related materials agrees with reports of other workers, except in degree. In the present study greater resistance was noted to Diazinon than to any other organophosphorus compound, whereas Weismann (1957) found that parathion tolerance was higher, although presumably the strain had not been exposed previously to this material.

It is interesting to note that the increase in tolerance to malathion was considerably less than to the other organophosphorous materials. Similarly, Metcalf has found that malathion-resistant flies were susceptible to parathion (Brown 1958). Also, Labrecque & Wilson (1957) reported that tolerances to Dipterex, parathion, and ronnel had increased only slightly in a field-collected strain which showed 37-fold resistance to malathion. Such findings are understandable since the materials involved, although all organophosphorous compounds, consist of three distinct types: phosphates, phosphorothioates, and phosphorodithioates.

The difference in degree of resistance between malathion and the phosphorothioates—Diazinon, Chlorthion, ronnel, and parathion—can be explained if resistance to Diazinon involves selection for individuals less active in thioate oxidation, for studies on the metabolism of such materials evidence that thioate and dithioate oxidations require different enzyme systems (Ball & Beck 1951, Metcalf & March 1953, Kok & Walop 1954, O'brien 1957).

In view of the structural dissimilarity between the phosphorothioates and dithioates, it seems reasonable to expect that different enzymes are involved in the hydrolysis of these two types of chemicals, in which case, if the mechanism of Diazinon-resistance is hydrolytic detoxification, rather than loss or reduction of thioate oxidation capacity, one would expect a higher degree of resistance to phosphorothioates than to dithioates. Preliminary toxicity studies by the writers indicate the increases in tolerance to other phosphorodithioates are even less than to malathion.

Cross-resistance to the carbamates, Pyrolan (10-fold), and Isolan (14-fold), was nearly as great as the resistance to some of the organophosphorous materials. Resistance to Sevin was even higher, although the exact degree could not be determined, since the solubility of the material was reached at concentrations below the LD50.

The high degree of cross-resistance to Sevin, Pyrolan, and Isolan would seem to preclude the use of carbamates of this type in the control of Diazinon-resistant flies. This conclusion, however, is not supported by Weismann's observations (1957) that Diazinon-resistant house flies in Switzerland could be effectively controlled by Isolan. Whether the strain's tolerance toward Isolan had changed apparently had not been determined, and thus there is inadequate evidence for assumption that the pathway to resistance was very different in Weismann's strain. However, his strain was characterized by a higher resistance to parathion than to Diazinon, which indicates at least minor differences between the two strains.

The mechanism of resistance to the carbamates may involve a hydrolytic detoxifying system with nonspecific substrate requirements. This premise would explain the greater resistance to Sevin, which is a relatively unstable carbamate.

Cross-resistance to Dilan appears to be a rather infrequent occurrence, this being the first report for organophosphorous-resistant strains. Although Dilan-resistance is accompanied by high cross-resistance to DDT, the reverse has not occurred (Metcalf 1955, Brown 1958, Fullmer & Hoskins 1951, March & Metcalf 1950) except in one strain characterized by resistance to knock-down by DDT (Winteringham 1952).

Nothing is known of the mechanism of resistance to Dilan. Kearns (1957) has indicated that Dilan will complex with DDT-dehydrochlorinase, but the importance of this as a resistance mechanism is uncertain, especially since most strains of DDT-resistant flies have not increased their tolerance to Dilan.

Rather low levels of cross-tolerance were exhibited to allethrins and pyrethrins, which is not unusual, for slight increases in pyrethrins-tolerance in fly strains resistant to chlorinated hydrocarbons have been observed repeatedly (Decker & Bruce 1952, Kocher *et al.* 1953, March 1952; Wilson & Gahan 1948, Blickle *et al.* 1948). The greatest degree of cross-tolerance to pyrethrins (5- 10-fold) was reported in a DDT-resistant strain which was characterized by resistance to knockdown (Busvine 1951, 1953). It may be well to point out that it was in such a strain that the unusual cross-resistance to Dilan was observed (Winteringham 1952). On the other hand, numerous instances are reported in which no cross-tolerance to pyrethrins was involved, either in chlorinated hydrocarbon-resistant strains (Goodwin-Bailey & Davis 1954, Busvine 1951, Pimental & Dewey 1950, March 1952, D'Allessandro *et al.* 1952) or in organophosphorous-chlorinated hydrocarbon-resistant strains (March *et al.* 1956).

Although the cross-resistance reported for pyrethrins and allethrin in this study is somewhat higher than that generally observed by previous workers, nothing suggests other than vigor tolerance.

The smallest increase in tolerance occurred with Lethane 384, the LD50 for the resistant strain being twice that for the normal line of flies. Actually, so small a change in susceptibility cannot be considered resistance in the same sense as the resistance displayed toward the chlorinated hydrocarbons, carbamates, and organophosphorous compounds, and is probably best termed a vigor tolerance or simply a normal difference between populations.

The absence of cross-resistance to thiocyanates is not surprising, for only a slight rise in Lethane 384-tolerance appeared in a BHC-resistant strain (Blickle *et al.* 1948) and no increase in tolerance in chlorinated hydrocarbon-resistant field (Busvine 1951) and laboratory strains (D'Allessandro *et al.*1952). The present findings are additionally interesting, however, since this instance involves resistance to a more heterogeneous group of chemicals. The facts suggest that more effective thiocyanates would offer promise in the control of resistant insects; however, the possibilities of developing more toxic thiocyanates

Table 3.—Effect of resistance on the regression coefficient for various insecticides.

Compound	Regression Coefficient		Ratio of Coefficients Resistant/ Susceptible
	Susceptible Strain	Resistant Strain	
Isolan	1.59	3.02	1.90
Allethrin	2.11	3.05	1.44
Pyrethrins	2.67	3.65	1.37
Dilan	3.24	4.25	1.31
Chlorthion	2.08	2.52	1.21
Ronnel	4.16	4.65	1.12
Pyrolan	1.94	1.79	0.92
Diazinon	3.84	3.46	0.90
Malathion	3.92	3.42	0.87
Parathion	4.20	3.19	0.76
Lethane 384	5.25	3.94	0.75
Dieldrin	2.56	1.00	0.39
Lindane	2.98	0.793	0.27
Chlordane	2.18	0.599	0.27

are not encouraging, inasmuch as more effective materials of this type have been unsuccessfully sought for many years.

Resistance to many of the materials tested was accompanied by either a positive or negative alteration in slope of the ld-p line, the latter being the more pronounced change (table 3 and figures 1 to 5). The largest decreases occurred for lindane, chlordane, and dieldrin, which also showed the greatest losses in effectiveness (excluding the DDT-type materials and Sevin, to which resistance was so high that ld-p lines could not be constructed).

Hewlett (1958) ascribes the decrease in slope which accompanies DDT-resistance to the relation between the amount of toxicant applied and that absorbed, and to variability among individuals in the quantity of DDT exerting a toxic effect at the site of action. This explanation is equally applicable to other insecticides for which there is a decrease in slope.

The increase in slope which was observed with several materials may be simply a reflection of increasing homogeneity of the strain with respect to response to the insecticide in question, or more specifically, a reduction in population variability with regard to the quantity of applied dose acting at the sensitive site. It is conceivable, however, that the percentage stored or detoxified per log dosage increment decreases more rapidly in the resistant strain, and if so this would be manifested as an increase in slope. It is unlikely that the alteration in slope is due to a change in the amount of toxicant absorbed, since such would necessarily involve an increase in the percentage absorbed per log dosage increment or at least less of a decrease than in the susceptible strain.

Resistance studies using laboratory-selected insects, even when the parent strain is from a wild resistant population, do not necessarily give an accurate representation of the problem as it exists in nature. There are numerous reasons for this, the two most important being the absence of environmental factors common to the natural habitat and the population limitations imposed by laboratory rearing.

In the present study, a comparison of toxicity (table 2) for five insecticides of dissimilar structure showed striking similarities both in the magnitude of resistance and the order of effectiveness between the field-laboratory-selected line and a wild resistant strain which received no additional selection in the laboratory. This fact indicates that the pattern of resistance determined for the laboratory-selected strain is identical, or nearly so, to one which exists under field conditions.

References Cited

Ascher, K. R. S. 1958. W.H.O. Inform. Circ. on Resistance Problem, No. 11, March 1958. Mimeo.

Ball, H. J., and S. D. Beck. 1951. The role of the circulatory and nervous systems in the toxic action of parathion. Jour. Econ. Ent. **44**(4): 558–64.

Blickle, R. L., A. Capelle, and W. J. Morse. 1948. Insecticide resistant house flies. Soap and Sanitary Chem. **24**(8): 139, 141, 149.

Brown, A. W. A. 1958. Insecticide resistance in Arthropods. W.H.O. Monogr. Ser. **38,** 240 pp.

Busvine, J. R. 1951. Mechanism of resistance to insecticides in houseflies. Nature **168**(4,266), 193–5.

Busvine, J. R. 1953. Forms of insecticide resistance in houseflies and body lice. Nature **171**(4,342), 118–9.

D'Allessandro, G., M. Mariani, and M. Gagliani. 1952. Ancora sulla resistenza della *Musca domestica* al DDT e ad alti insetticidi clorurate, selezione di ceppi formiti di caratteristicke differenti da unica popolazione catturata in natura. Riv. Parissiti **13:** 169–75.

Decker, G. C., and W. N. Bruce. 1952. House fly resistance to chemicals. Amer. Jour. Trop. Med. Hyg. **1:** 395–403.

Fullmer, O. H., and W. M. Hoskins. 1951. Effects of DDT upon the respiration of susceptible and resistant house flies. Jour. Econ. Ent. **44**(6): 858–70.

Goodwin-Bailey, K. F., and M. Davis. 1954. Occurrence of dieldrin-resistance in wild *Musca domestica* L. in England. Nature **173**(4,396): 216–7.

Hansens, E. J. 1953. Failure of residual insecticides to control house flies. Jour. Econ. Ent. **46**(2): 246–8.

Hansens, E. J. 1958. House fly resistance to Diazinon. Jour. Econ. Ent. **51**(4): 497–9.

Harrison, C. M. 1950. DDT-resistance in an Italian strain of *Musca domestica*. Bull. Ent. Res. **42:** 761–8.

Hewlett, P. S. 1958. Interpretation of dosage-mortality data for DDT-resistant house flies. Ann. Appl. Biol. **46**(1): 37–46.

Hoskins, W. M., and H. T. Gordon. 1956. Arthropod resistance to chemicals. Ann. Rev. Ent. **1:** 89–122.

Kearns, C. W. 1957. W.H.O. Inform. Circ. on Resistance Problem, No. 9, November 1957. Mimeo.

Keiding, J. 1956. Resistance to organic phosphorous insecticides of the house fly. Science **123**(3,209): 1173–4.

Kocher, C., W. Roth, and J. Treboux. 1953. Die Bekämpfung resistenter Stubenfliegen (*Musca domestica* L.) mit Diazinon. Anz. Schadlingsk. **26:** 65–71.

Kok, G. C., and J. N. Walop. 1954. Conversion of *O,O*-diethyl-*O*-p-nitrophenyl thiophosphate (parathion) into an acetylcholinesterase-inhibitor by the insect fat body. Biochim. et Biophys. Acta **13:**510–5.

Labrecque, G. C., and H. G. Wilson. 1957. House fly resistance to organophosphorous compounds. Agric. Chem. **12**(9): 46–7, 147, 149.

Labrecque, G. C., H. G. Wilson, and J. B. Gahan. 1958. Resistance of house flies in Florida to organophosphorous insecticides. Jour. Econ. Ent. **51**(5): 616–7.

March, R. B. 1952. Summary of research on insects resistant to insecticides. Nat. Res. Counc. Publ. No. 219, Conference on insecticide resistance and insect physiology, pp. 45–53. Dec. 8–9, 1951.

March, R. B., L. L. Lewallen, and R. L. Metcalf. 1956. A

summary of investigations with organophosphorous insecticides for the control of insecticide-resistant houseflies in southern California. Unpublished working document WHO/Insecticides/59, 17 pp.

March, R. B., and R. L. Metcalf. 1950. Insecticide resistant flies. Soap and Sanitary Chem. 26(7): 121–5, 139.

Meltzer, J. 1956. Multiresistentie bij de kamervlieg, *Musca domestica* L., opgewekt door selectie met insecticiden. Meded. Landbouw. Opzoek. Staat Gent 21(3): 459–82.

Metcalf, R. L. 1955. Physiological basis for insect resistance to insecticides. Physiol. Rev. **35:** 197–232.

Metcalf, R. L., and R. B. March. 1953. Further studies on the mode of action of organic thionophosphate insecticides. Ann. Ent. Soc. America 46(1): 63–74.

O'brien, R. D. 1957. Properties and metabolism in the cockroach and mouse of malathion and malaoxon. Jour. Econ. Ent. 50(2): 159–64.

Pimental, D., and J. E. Dewey. 1950. Laboratory tests with house flies and house fly larvae resistant to DDT. Jour. Econ. Ent. 43(1): 105.

Pimental, D., H. H. Schwardt, and J. E. Dewey. 1953. Development and loss of insecticide resistance in the house fly. Jour. Econ. Ent. 46(2): 295–8.

Schoof, H. F., and J. W. Kilpatrick. 1958. House fly resistance to organophosphorous compounds in Arizona and Georgia. Jour. Econ. Ent. 51(4): 546.

Weismann, R. 1956. W.H.O. Inform. Circ. on Resistance Problem, No. 3, November 1956. Mimeo.

Weismann, R. 1957. W.H.O. Inform. Circ. on Resistance Problem, No. 8, September 1957. Mimeo.

Wilson, H. G., and J. B. Gahan. 1948. Susceptibility of DDT-resistant house flies to other insecticidal sprays. Science **107:** 276–7.

Winteringham, F. F. W. 1952. Metabolism of DDT by resistant house flies. Natl. Res. Counc. Publ. No. 219, Conference on insecticide resistance and insect physiology. pp. 61–5. December 8–9, 1951.

Insecticidal Activity of the Enantiomorphs of *O*-Ethyl *S*-2-(Ethylthio)-ethyl Ethylphosphonothiolate[1,2,3]

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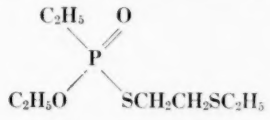
ABSTRACT

The *dextro*, *levo* and racemic forms of *O*-ethyl *S*-2-(ethylthio)ethyl ethylphosphonothiolate were prepared and examined for insecticidal properties. The *levo* isomer was about 10 times more toxic to certain insects than the *dextro* isomer. Also, the *levo* isomers inactivated fly-brain cholinesterase 11 times faster than the *dextro* isomer.

In a recent paper by Aaron *et al.* (1958b) the synthesis of the enantiomorphs of *O*-ethyl *S*-2-(ethylthio)ethyl ethylphosphonothiolate is described. The enantiomorphs of this compound were prepared directly



from the *dextro* and *levo* forms of *O*-ethyl ethylphosphonothioic acid which were previously described by Aaron *et al.* (1958a). These workers determined the velocity constants for the inactivation of cholinesterase from different sources by *O-d,l-*, *d-* and *l*-ethyl *S*-2-(ethylthio)ethyl ethylphosphonothiolate and found that the *levo*-isomer inactivated cholinesterase 10 to 20 times faster than the *dextro*-isomer.

In connection with our work on the structure and insecticidal activity of esters of alkyl alkylphosphonic acids we prepared and examined the properties of *O-d,l*-ethyl *S*-2-(ethylthio)ethyl methyl-, ethyl- and *n*-propylphosphonothiolate (Fukuto & Metcalf 1959, Fukuto *et al.* 1959). These compounds showed high activity against a wide variety of insects both as contact and as systemic insecticides. In view of the large difference in the cholinesterase inhibition rate between the *dextro-* and *levo-*isomers of the ethyl analog reported by Aaron *et al.* (1958b) it was decided to examine the activity of these isomers against insects.

MATERIALS AND METHODS.—*O-d,l*-Ethyl *S*-2-(ethylthio)ethyl ethylphosphonothiolate was prepared according to the procedure of Fukuto & Metcalf (1959) from ethyl ethylphosphonochloridate and 2-(ethylthio)ethanethiol. The product distilled at 96–98 (0.1 mm.), n_D^{30} 1.5086.

O-d- and *l*-Ethyl *S*-2-(ethylthio)ethyl ethylphosphonothiolate were prepared according to the procedure of Aaron *et al.* (1958b) from the sodium salt of *O-d-* and *l*-ethyl ethylphosphonothioic acid and 2-chlorodiethyl sulfide. The *dextro*-isomer distilled at 108 (0.3 mm.), n_D^{30} 1.5060, [α] D $-47.4°$ ($\alpha_{OBS} -0.79°$, acetone, 1 dcm., *C* 1.668). The *levo*-isomer distilled at 90–92 (0.05 mm.), n_D^{30} 1.5060, [α] D $+44.1°$ ($\alpha_{OBS} +0.87$, acetone, 1 dcm., *C* 1.974).

O-d,l-Ethyl Ethylphosphonothioic acid.—Although the separation of *O-d,l*-ethyl ethylphosphonothioic acid into its enantiomorphs is described by Aaron *et al.* (1958a) the preparation of the *d,l*-acid itself only recently appeared in the literature (Hoffman *et al.* 1959). This acid was prepared as follows: To a stirred solution of 12.2 gm. *O*-ethyl ethylphosphonochloridothionate in 50 ml. ethyl alcohol was added dropwise an aqueous solution containing 5.7 gm. sodium hydroxide. The reaction mixture was cooled in ice during the addition and then stirred at room temperature for 30 minutes. The sodium chloride which separated was removed by filtration and the filtrate concentrated to a solid. The residual solid was dissolved in a small amount of water, acidified with concentrated hydro-

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chloric acid and the product extracted into chloroform. The chloroform solution was dried over anhydrous sodium sulfate and the phosphonothioic acid was distilled, b.p. 74–77°C. (0.2 mm.), n_D^{25} 1.4865. The yield was 4.6 g., 43% of theoretical. The dicyclohexylamine salt melted at 160–162° C.

The preparation of *O*-ethyl ethylphosphonochloridothionate has been described by Hoffman *et al.* (1958). These authors reacted *O*-ethyl ethylphosphonothioic dichloride with ethyl alcohol in the presence of triethylamine in benzene. Our method of synthesis differed slightly in that *O*-ethyl ethylphosphonothioic dichloride was treated directly with one equivalent of sodium ethoxide in ethyl alcohol. The product thus obtained distilled at 72–76° C. (10 mm.) n_D^{28} 1.4895.

The *O*-*d*,*l*-ethyl ethylphosphonothioic acid was resolved *via* the quinine salt according to Aaron *et al.* (1958a).

The rate of reaction of *O*-*d*,*l*-, *d*-, and *l*-ethyl *S*-2-(ethylthio)ethyl ethylphosphonothiolate with house-fly-brain (*Musca domestica* L.) cholinesterase was determined manometrically at 37.5° by the method described by Aldridge (1950), using double-armed Warburg flasks. The reactions were carried out in a mixture consisting of a brei of three homogenized fly heads per ml. of a solution 0.025 *M* in sodium bicarbonate, 0.15 *M* in sodium chloride, 0.04 *M* in magnesium chloride, and 0.01 *M* in acetylcholine bromide.

The techniques for determination of the contact toxicity to the common house fly, mosquito larvae (*Culex quinquefasciatus* Say), and honey bee (*Apis mellifera* L.) have been described (Metcalf & March 1949, 1953).

DISCUSSION OF RESULTS.—The inactivation of fly-brain cholinesterase by *O*-*d*,*l*-, *d*- and *l*-ethyl *S*-2-(ethylthio)ethyl ethylphosphonothiolate was determined at 37.5° C. The values for the rate constants for inactivation (Ke in liter/mole·min.) are given as follows: *dextro*-isomer 6.7×10^4, *levo*-isomer 7.6×10^5 and *d*,*l*-mixture 4.4×10^5. These values are in good agreement with those found by Aaron *et al.* (1958b) for the inactivation of cholinesterases from four different sources. For the inactivation of electric eel cholinesterase at 25° C. they found the following rate constants: *dextro*-isomer 4.7×10^3, *levo*-isomer 8.5×10^4, and *d*,*l*-mixture 4.6×10^4 liter/mole·min. From the activation energies of 12.8 and 11.5 Kcal/mole for eel cholinesterase inactivation by the *d*- and *l*-isomers the rate constants at 37.5° C were calculated and found to be 1.1×10^4 liter/mole·min. for the *d*-isomer and 1.9×10^5 liter/mole·min. for the *l*-isomer. It appears from these data that electric eel cholinesterase is inactivated about one-fifth as fast as fly-brain cholinesterase.

The rate constants given above show that the *levo*-isomer reacts 11 times faster with fly-brain cholinesterase than the *dextro*-isomer and 1.8 times faster than the *d*,*l*-mixture. The rate constant found for the *d*,*l*-mixture is very close to the mean of the two resolved forms and is proof of the purity of this compound. This is of interest since the racemic mixture was prepared by an independent method of synthesis.

The data given below show the contact toxicity of the *d*, *l*-, *d*- and *l*-isomers to the common house fly, mosquito larvae, and honey bee.

Isomer	LD_{50} *House Fly* (γ/*gm.*)	LC_{50} *Mosquito Larvae* (*p.p.m.*)	LD_{50} *Honey bee* (γ/*gm.*)
d, *l*	9.5	0.15	—
d	30.0	0.80	6.6
l	5.0	0.08	0.68

These data show that the *levo*-isomer is 6, 10 and 9.7 times more toxic to the house fly, mosquito larvae, and honey bee, respectively, than the *dextro*-isomer. As expected, the toxicity of the *d*,*l*-mixture was slightly better than half the *l*-isomer. It is clear that the toxicity of these compounds closely parallels the cholinesterase inactivation rates.

In order to compare the rate of lethal action of the *d*- and *l*-isomers, 5λ of each compound was distributed uniformly in a petri dish and 20 flies placed in each dish. The number of flies knocked down at different time intervals were recorded as follows:

Time (*min.*)	*l*	*d*
3	15	0
5	20	2
8	—	8
9	—	15
11	—	20

The superiority of the *levo*-isomer over the *dextro*-isomer is also demonstrated here.

REFERENCES CITED

Aaron, H. S., T. M. Shryne, and J. J. Miller. 1958a. The stereochemistry of asymmetric phosphorus compounds. I. The resolution of *O*-ethyl ethylphosphonothioic acid. Jour. Amer. Chem. Soc. **80:** 107–10.

Aaron, H. S., H. O. Michel, B. Witten, and J. J. Miller. 1958b. The stereochemistry of asymmetric phosphorus compounds. II. Stereospecificity in the irreversible inactivation of cholinesterases by the enantiomorphs of an organophosphorus inhibitor. Jour. Amer. Chem. Soc. **80:** 456–8.

Aldridge, W. N. 1950. Some properties of specific cholinesterase with particular reference to the mechanism of inhibition by diethyl *p*-nitrophenyl thiophosphate (E605) and analogues. Biochem. Jour. **46:** 451–460.

Fukuto, T. R., and R. L. Metcalf. 1959. The effect of structure on the reactivity of alkylphosphonate esters. Jour. Amer. Chem. Soc. In Press.

Fukuto, T. R., R. L. Metcalf, and M. Y. Winton. 1959. Insecticidal properties of phosphonic acid esters. Unpublished.

Hoffmann, F. W., D. H. Wadsworth, and H. D. Wiess. 1958. Organic phosphorus compounds. III. *O*,*O*-Dialkyl alkylphosphonothioates and *O*-alkyl alkylphosphonochloridothioates. Jour. Amer. Chem. Soc. **80:** 3945–8.

Hoffmann, F. W., B. Kagan, and J. H. Canfield. 1959. Organic phosphorus compounds. V. The preparation of *O*-alkyl alkylphosphonothioic acids. Jour. Amer. Chem. Soc. **81:** 148–51.

Metcalf, R. L., and R. B. March. 1953. The isomerization of organic thionophosphate insecticides. Jour. Econ. Ent. **46:** 288–94.

Metcalf, R. L., and R. B. March. 1949. Studies of the mode of action of parathion and its derivatives and their toxicity to insects. Jour. Econ. Ent. **42:** 721–8.

Demeton Residues on Turnips, Squash, Tomatoes, Peppers and Snap Beans[1]

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Abstract

The systemic pesticide demeton penetrates into the edible portions of vegetables in varying degrees. Little, if any, toxicant was found to be translocated into developing turnip roots, squash, tomatoes, and peppers as a result of foliar applications. Demeton-treated turnip tops contained initial residues high enough to require at least 21 days' weathering and growth to reach tolerance levels. Snap beans, having a lower tolerance, required a 14-day interval between last application and harvest to satisfy Federal requirements.

Demeton is a systemic insecticide that is applied to crops to control sucking pests such as aphids, mites, and mealy bugs. Unfortunately, the normal weathering processes are generally not so effective in lowering toxic residue levels of plant-metabolized systemics as are conventional surface residues. Therefore, considerable investigation is necessary to determine the concentrations of this class of insecticide that remain on or in treated raw agricultural commodities prepared for interstate commerce. The Food and Drug Administration has established a tolerance of 0.75 p.p.m. of demeton on numerous vegetables and a 0.3 p.p.m. tolerance on beans. To date, no official tolerance has been established on turnips, squash, tomatoes, or peppers.

Unfortunately very little published information is available on demeton residues *per se* remaining on and in vegetable crops after a normal spray program. Chemagro Corporation, New York, has on file numerous unpublished demeton residue reports that were used to support their tolerance requests. Butler *et al.* (1957) report demeton residue results on various fruits, vegetables, and forage crops from the Pacific Northwest. Rates of decomposition of the thiol and thiono metabolites of demeton in citrus pulp and bean and cotton leaves are reported by Metcalf *et al.* (1955). Previously Van Middelem & Waites (1958) noted that a 21-day waiting period was sufficient following foliar applications of demeton on collards, lettuce, and mustard, provided the recommended dosage applications were spaced 2 or 3 weeks apart. Wilcox & Howland (1956) found residues of demeton below tolerance levels 5 days after application of 6- and 11-ounce dosages per acre. Wilcox & Howland (1957) reported that at least a 21-day interval should be allowed on lima beans between last application and harvest to approach the 0.3 p.p.m.-tolerance level.

Method.—The analytical procedure used in these studies is essentially an enzymatic method adapted as a micro technique necessary for residue analyses by Giang & Hall (1951). Later Hensel *et al.* (1954) modified the procedure so that subsurface as well as surface residues could be determined. This enzymatic procedure utilizes the property of some organic phosphates, including demeton, to inhibit the enzyme cholinesterase.

Aqueous extracts of various demeton-treated homogenates were subjected to the action of cholinesterase using human blood plasma as a source. The inhibitory action of demeton on cholinesterase can be expressed schematically by the following reaction:

$$\text{Demeton} + \text{cholinesterase} \rightarrow \text{partially or completely inactivated cholinesterase}$$

$$\text{Cholinesterase} + \text{acetylcholine} \rightarrow \text{acetic acid} + \text{choline}$$

Since the enzymic or hydrolysis reaction is a rate process, an increase in either cholinesterase or acetylcholine-chloride will increase the amount of acetic acid formed per unit length of time. The acetic acid released by the hydrolytic reaction in the presence of a buffer results in a linear relationship between the liberated acetic acid and pH change (delta pH) measured. Therefore, partially inactivated cholinesterase (treated with demeton) will hydrolyze a smaller amount of acetic acid and consequently a smaller delta pH will result. The per cent inhibition or inactivation, as measured by delta pH, is proportional to the demeton toxicant present, when concentration is plotted logarithmically and per cent inhibition is plotted linearly.

Procedure.—All crops were grown in randomized block field experiments designed specifically for residue studies. Foliar applications were made at the rate of 100 gallons per acre with a John Bean Hi-Lo sprayer applying the dosage rates specified in tables 1 to 5. Soil applications of demeton were made by pouring 1 pint of the water emulsion (containing 8 ounces actual ingredient per 100 gallons) around the base of the pepper and tomato plants at the time of transplanting. The original formulation used in both the foliar and soil applications was a 26% emulsifiable concentrate.

On sampling dates all vegetables were harvested randomly from field plots, brought to the laboratory, cut, and mixed. Representative 1,000-gram samples were selected, placed in polyethylene bags, and frozen. All samples were kept in the freezer until date of analysis.

Before analysis, samples were thawed, macerated in blender, and then pressed through four layers of cheesecloth. The resultant juices were collected and brought to pH of 7.0. Immediately 0.75 ml. of cholinesterase was added, mixed, and diluted to volume with untreated crop juice in a 10-ml. volumetric flask.

After a 70-minute incubation period at 37.5° C., a 5-ml. aliquot was pipetted and transferred to a 15-ml. beaker. The solution was then buffered to pH 8.0 and placed in controlled temperature water bath for 10 minutes to attain equilibrium. At this time the initial pH reading was made, followed by the addition of 0.5 ml. acetylcholine chloride to each beaker. After 2 hours in the 37.5° C.-controlled temperature water bath, the final pH reading was made in the solution constantly stirred by a magnetic stirring bar. The difference (delta pH) between the initial

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Table 1.—Residues of demeton on and in turnip greens and roots at various intervals after application (average three replications in p.p.m.).[a]

Plant Part	Ounces of Active Ingredient/A.	Time Lapse Since Last Foliar Application					
		4 Hrs.	1 Day	3 Days	8 Days	15 Days	22 Days
Greens	2	1.7	2.3	3.0	6.4	3.6	0.7
	4	3.8	10.0	7.4	6.4	5.9	1.6
Roots	4				0.1	0.1	

[a] Climatological data:
Dec. 9–31, 1954—rainfall 1.50″—mean temp. 54.3° F.
Jan. 1–25, 1955—rainfall 3.91″—mean temp. 57.1° F.
Application dates:
Dec. 9, 20, 27, 1954, Jan 3, 1955.

and final pH of the untreated vegetable sample was used to calculate all demeton-treated samples and represents an uninhibited system. To calculate the per cent inhibition of the demeton-treated samples the following formula was used:

$$\% \text{ inhibition} = \frac{(\text{delta pH of check sample}) - (\text{delta pH of treated sample})}{\text{delta pH of check sample}} \times 100$$

RESULTS.—*Turnip Tops and Roots* (table 1).—After four foliar applications of demeton the residues appeared to build up in the turnip greens until approximately 8 days after the fourth spray. Subsequent samples indicated that the residues disappeared at a relatively constant rate until the last sampling date, 22 days after treatment. The 4-ounce active dosage resulted in residues considerably higher than the 2 ounce-dosage 2 and 3 weeks after treatment. Results shown in table 1 indicate that, despite the higher dosage rate, the turnip roots contained only a trace of demeton even 8 days after treatment (when the foliage residues were at their peak).

Squash (table 2).—After three sampling dates (the first two 1 week after the initial and second applications and the third only 2 days after the last application), only trace amounts of demeton were found on or in the squash. It can be assumed that very little, if any, of the demeton applied on the foliage had been translocated into the rapidly developing fruit.

Table 2.—Residues of demeton on and in squash at various intervals after application (average of three replications in p.p.m.).[a]

Ounces of Active Ingredient/A.	No. of Applications	Days After Last Foliar Application	Residue
2	1	7	0.00
4		7	.00
2	2	6	.02
4		6	.04
2	3	2	.05
4		2	.10

[a] Climatological data:
May 10–26, 1955—rainfall 1.63″—Mean temp. 79.2° F.
Application dates:
May 10, 17, 24, 1955.

Table 3.—Residues of demeton on and in tomatoes at various intervals after application (average of three replications in p.p.m.).[a]

Ounces of Active Ingredient/A.	Time Lapse After 4th Foliar Application	Residue (p.p.m.)	Ounces of Active Ingredient/A.	Days After Last Application	Residue (p.p.m.)
	1955 Test			*1957 Test*	
1	24 hrs.	0	8	28 (foliar)	0.17
2	24 hrs.	0	8	35 (soil)	.43
1	3 days	0			
2	3 days	0		*1958 Test*	
1	7 days	0	8	25 (foliar)	0.11
2	7 days	0	8	40 (soil)	.14
1	14 days	0		50 (soil)	.09
2	14 days	0.1		60 (soil)	.00

[a] Climatological data:
1955 test—May 10–31 —rainfall 1.91″—mean air temp. 79.0° F.
1955 test—June 1–14 —rainfall 1.79″—mean air temp. 77.4° F.
1957 test—May 1–June 4 —rainfall 6.27″—mean air temp. 73.0° F.
1958 test—May 2–June 11—rainfall 5.70″—mean air temp. 75.8° F.
1958 test—May 2–July 3—rainfall 10.90″—mean soil temp. 82.7° F.
Foliar applications:
May 10, 17, 24, 31, 1955.—May 1, 9, 1957.—May 2, 19, 16, 1958.
Soil applications:
May 1, 1957, and May 2, 1958.

Tomatoes (table 3).—As a result of 3-year data it is quite apparent that little or no demeton is being translocated to the maturing fruit as a result of repeated foliar applications. There is some indication that small quantities of demeton are being carried to the fruit as a result of one soil application at transplanting time. However, after an interval of 6 weeks the only significant concentrations of the systemic were found in the maturing fruit (green ripe stage).

Peppers (table 4).—The demeton residues found on and in mature peppers 1 week after repeated foliar applications appeared relatively insignificant. However, as a result of one soil application at transplanting time, relatively high residues appeared to exist in mature peppers as long as 6 weeks after this treatment.

Snap Beans (table 5).—Results of 2 years' data (three foliar applications at 10-day intervals) indicated that relatively small but above-tolerance levels of demeton remained on snap beans 3 to 7 days after treatment. It would appear that most of the systemic residue found on the bean pod was due to direct spray contact and did not result from any significant translocation from the foliage.

Table 4.—Residues of demeton on and in peppers at various intervals after application of 8 ounces of the active ingredient per acre (average of three replications in p.p.m.).[a]

Time Lapse After Last Application	Residue (p.p.m.)	Days After Last Application	Residue (p.p.m.)
1957 Test		*1958 Test*	
4 hrs. after 3rd foliar	0.45	7 after 6th foliar	0.39
6 days after 3rd foliar	.30	14 after 6th foliar	.28
14 days after 3rd foliar	.17	20 after 6th foliar	.17
21 days after 3rd foliar	.11		
21 days after one soil	1.28	50 after one soil	0.15
27 days after one soil	1.25		
35 days after one soil	1.01	57 after one soil	.10
42 days after one soil	0.74	63 after one soil	.11

[a] Climatological data:
1957 Test—May 1–June 4—rainfall 6.27″—mean air temp. 73.0° F.
1958 Test—May 2–30 —rainfall 5.54″—mean air temp. 74.7° F.
1958 Test—June 1–July 3—rainfall 4.91″—mean air temp 80.5° F.
1958 Test—May 2–July 3—rainfall 10.90″—mean soil temp. 82.7° F. (4-inch depth)
Application dates:
May 1, 9, 21, 1957 (foliar), May 1, 1957 (soil).
May 2, 9, 23, 29, June 4, 13, 1958 (foliar), May 2, 1958 (soil).

Table 5.—Residues of demeton on and in snap beans at various intervals after application (average of three replications in p.p.m.).[a]

Test Year	Ounces of Active Ingredient/A.	Time Lapse Since Last Application			
		4 Hrs.	3 Days	7 Days	14 Days
1956	2	0.34	0.24	0.20	
	4	.53	.40	.38	
1957	8	.54		.39	0.30

[a] Climatological data:
1956 Test—Apr. 27–May 28 rainfall 4.52"—mean temp. 75.7° F.
1957 Test—May 1–June 4 rainfall 6.27"—mean temp. 73.0° F.
Application dates:
Apr. 27, May 3, 14, 1956 (foliar) May 1, 9, 21, 1957 (foliar).

DISCUSSION.—It is not the purpose of this publication to become involved in all the possible physiological ramifications presented by the use of a systemic insecticide such as demeton on actively growing vegetables. However, a very limited discussion on some of the general physiological, chemical, and climatological aspects regarding systemic insecticides might prove helpful in explaining the differences in residues obtained.

Emphasis should be placed on the fact that the commercial insecticide demeton (Systox) is composed of two isomers: *O,O*-diethyl O-2 ethyl mercapto ethyl thionophosphate (thiono isomer) and *O,O*-diethyl S-2 ethyl mercapto ethyl thiophosphate (thiol isomer). The thiol isomer, although only slightly water soluble, is about 10 times more so than the thiono isomer. Moreover, the anticholinesterase activity of the thiol isomer is almost 100 times greater than that of the thiono isomer. Isomerization occurs from the thiono to the thiol isomer, and at 130° F. approximately 70% isomerization occurs in 3 hours. The commercial combination of thiono and thiol isomers is approximately a 65:35 ratio respectively.

Usually in the experiments reported the systemic was applied only as a foliar application. However, one soil-drench application at transplanting time was used in several treatments and the relative efficiency and persistency of the resultant demeton residues were noteworthy.

Systemic insecticides, such as demeton, can enter the interior of plants via the leaves, roots, or stems. The roots, however, offer the most efficient entrance into the plant. Once the systemic is absorbed by the roots, it is often readily translocated upward in the transpiration stream to the most rapidly growing portions of the plant. While leaves may not be considered the normal absorptive areas of a plant, limited absorption of demeton does occur under ideal conditions. When a systemic insecticide is applied to plant foliage, numerous processes such as evaporation, absorption, and breakdown may occur simultaneously at varying rates. The quantity of insecticide lost to the atmosphere by evaporation depends largely on the vapor pressure of the insecticide and the weather conditions at time of application. With high temperature and air movement evaporational losses may be considerable. Evaporation can be an extremely important factor in the behavior of systemic insecticides when applied to aerial portions of the plant. If the vapor is toxic to insects (as with demeton) a high initial kill may result by fumigant action on certain crops under favorable conditions. However, the considerable loss of toxicant by evaporation often significantly reduces the quantity available for absorption and subsequent systemic action. Of course, once the insecticide becomes truly systemic, it often requires a considerably longer period for the plant's metabolic breakdown processes to detoxify the demeton, in comparison with the normal decomposition pattern of a conventional plant surface-applied organic phosphate.

The turnip experiment showed, despite repeated foliar applications of demeton, that very little, if any, of the systemic was being translocated from aerial portions to the roots. There is a distinct possibility, however, that some of the foliar-applied systemic fell on the soil and was eventually absorbed by the roots and translocated to the aerial portions of the turnip plant. This is substantiated by a gradual buildup of residues in the turnip tops until 8 days after treatment. Although the squash received three consecutive weekly foliar applications, only trace quantities of demeton could be determined in the squash itself at sampling time. Care was taken to apply the demeton while the squash was relatively immature and in a rapid stage of development. (These conditions are ideal for translocation of a systemic toxicant.)

Only trace amounts of demeton could be detected in the relatively mature tomato fruit that had received four weekly foliar applications. That very little if any of the systemic had been translocated from the foliage into the developing fruit may be concluded. The trace quantities picked up on some of the fruit was probably caused by direct contamination from the foliar sprays. However, one soil application on the tomatoes at transplanting time resulted in some accumulation of the toxicant in the green ripe fruit at least 5 weeks after treatment.

No significant amount of demeton translocation from three foliar applications (10 days apart) was apparent on mature peppers. The residues observed appeared to be the result of direct contamination from the previous foliar applications. However, the one soil application resulted in above-tolerance residues in the pepper itself as long as 6 weeks after treatment in the 1957 test. Results of the 1958 test would indicate that demeton residues in the fruit were down to insignificant levels in the mature pepper 7 weeks after the transplant water treatment to the soil.

Although the snap beans received 3 to 4 foliar applications (10 days apart), demeton residues were not excessive and probably were caused entirely by direct contamination from the foliar sprays. It is assumed that little or no translocation was involved in the demeton residues found on or in the edible bean pod.

CONCLUSIONS.—As a result of the data reported, there is evidence that 7 days is a safe interval following the last demeton foliar application to harvest turnip roots, squash, tomatoes, and peppers following three 4-ounce active dosages spaced 7 to 10 days apart. However, such is not the case with the foliar-treated turnip greens, which require at least a 21-day interval between last application and harvest to be safe from excess demeton residues.

Although the demeton residues determined on snap beans were considered relatively low, a 14-day waiting period following the last foliar application is recom-

mended. The longer interval is required because of the lower demeton tolerance set by the Federal Food and Drug Administration on beans.

Apparently an entirely new set of conditions is involved in the soil treatments of demeton. Generally speaking, it is not a safe practice residuewise to apply demeton to the soil beyond the transplant water stage of plant growth. It appears, since the roots are the natural avenue of demeton penetration, that once the toxicant is absorbed, it is rather efficiently translocated to distant aerial plant parts that are in an active stage of development.

REFERENCES CITED

Butler, L. I., and W. E. Westlake. 1957. Demeton residues on fruits, vegetables, and forage crops. Jour. Econ. Ent. **50**(6): 737–9.

Giang, P. A., and S. A. Hall, 1951. Enzymatic determination of organic phosphorus insecticides. Analyt. Chem. **23**(12): 1830–34.

Hensel, J., A. N. Hewett, U. N. Sheets, and R. C. Scott. 1954. Microestimation of demeton residues. Chemagro Corporation, N. Y., N. Y., and Pittsburgh Coke and Chemical Co. Research Laboratoriees, Pittsburgh, Pa. Unpublished mimeo, 13 pp.

Metcalf, R. L., R. B. Marsh, T. R. Fukuto, and M. G. Maxon. 1955. The nature and significance of systox residence in plant materials. Jour. Econ. Ent. **48**(4): 364–9.

Van Middelem, C. H., and R. E. Waites. 1958. Demeton residues in collards, lettuce and mustard. Jour. Agric. Food Chem. **6**(8): 594–7.

Wilcox, J., and A. F. Howland. 1956. Control of two-spotted spider mite on strawberries. Jour. Econ. Ent. **49**(2): 242–4.

Wilcox, J., and A. F. Howland. 1957. Experiments for the control of spider mites on lima beans. Jour. Econ. Ent. **50**(2): 128–32.

Blacklight as an Attractant to European Chafer Beetles[1]

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ABSTRACT

Fluorescent blacklight that radiates energy at 3,200 to 3,800 angstroms was highly attractive to adults of the European chafer *Amphimallon majalis* (Raz.). Traps containing these lights captured up to 70 times as many beetles as the most attractive chemically baited traps when exposed to very low populations late in the 1958 season. Light-traps caught beetles on nights when none were seen in flight. Chemically baited traps captured beetles only during a 30-minute period at sundown, whereas beetles were captured by light traps during a 9-hour period throughout the night. Chemically baited traps require an open situation, but light-traps were more efficient when placed near trees to which beetles flew. Contrary to the action of chemically baited traps, light-traps caught more females than males.

Although Tashiro and Fleming (1954) demonstrated that chemically baited traps are as effective as visual scouting for detecting light infestations of the European chafer, *Amphimallon majalis* (Raz.), the visual method is still more common. During the last 2 or 3 years several improvements have been made in these traps, but they are still not sufficiently efficient for the expanding survey operations.

Taylor *et al.* (1956) reported that fluorescent lamps, designated in the trade as BL (blacklight), which radiate energy in the near ultraviolet region (3,200 to 3,800 angstroms), have attracted almost all economic species known to be photopositive. Since the scarabaeids *Cyclocephala borealis* Arrow and *Phyllophaga* spp. were listed, experiments were conducted at Geneva, New York, to determine the reaction of the European chafer to these lights.

Three modified omnidirectional traps (fig. 1, center) with baffles attached were used. An 18 inch-long 15-watt blacklight lamp (General Electric F 15T8/BL) was the attractant. The lamp was equipped with a ballast and starter of standard fluorescent type and operated on 110–125 volt a.c. Each sheet metal trap was 14 inches wide and 40 inches long, and weighed approximately 16 pounds. These traps, provided by the Agricultural Engineering Research Division, are hereafter referred to as the AE traps.

Since the AE traps were received too late in 1958 for testing during the main flight of adult chafers, they were exposed only to very light flights. The limited but encouraging results obtained from July 21 through September 2 are reported herein. The most efficient chemically baited trap developed at the time served as the basis for comparison. This plastic trap (fig. 1, left), furnished by the Plant Pest Control Division, was baited with a 3:1 Java citronella-eugenol mixture. The baffles were enclosed by 1-inch hexagonal-weave poultry netting.

All the traps were suspended from standards which stood approximately 6 feet high. They were placed in the same lot of approximately 1 acre at Phelps, New York. The lot was surrounded by trees and buildings and was in direct view of many incandescent street lights. It is not known whether these lights affected the activity of any of the traps.

EXPLORATORY EXPOSURE TESTS.—Adult chafers are normally in flight from mid-June to late July, with a few stragglers appearing beyond this period. The beetles leave the ground between 8:30 and 9 P.M. (Eastern daylight time), and fly to nearby trees. After a brief swarming period, with peak activity about 9 P.M., they come to rest in the trees to mate. They return to the soil before dawn. The beetles are attracted to the chemically baited traps for only a 30-minute period during the flights at sundown.

Since red had been found to be the most attractive color for chemically baited traps (Tashiro & Fleming 1954), the light-traps were also painted red. When the

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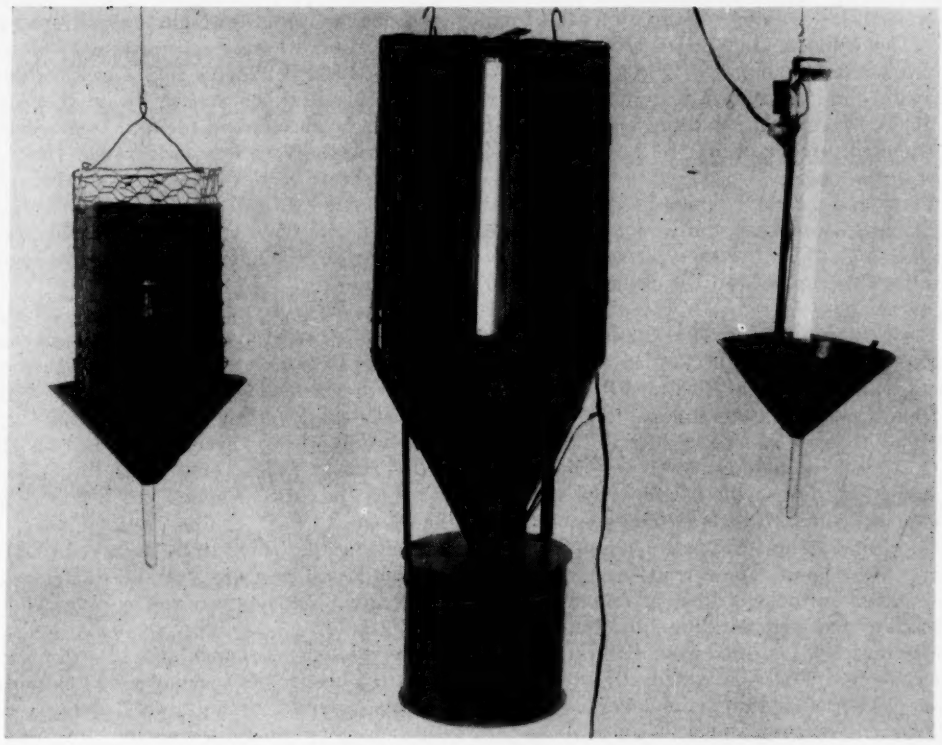

FIG. 1.—Traps used for capturing European chafer adults. Left—plastic trap with Java citronella-eugenol; center—omnidirectional A.E. light-trap with 15-watt fluorescent blacklight; right—Geneva modification of light-trap with 15-watt fluorescent blacklight.

first observations were made during the evening flight, they did not attract any more beetles than the chemically baited traps. However, when the light-traps were operated all night, they caught many more beetles. It was assumed then that most of the beetles were attracted to the lights on their return to the ground.

After it became apparent that lights did show some attraction to beetles, two of the AE traps were exposed with illumination all night; Java citronella-eugenol was added to one. The third was exposed unlighted, but was baited with the chemical. It was evident that the light was far more attractive than the chemical and that the bait added nothing to the efficiency of the trap. After 3 nights' exposure, the trap with the light only caught 85 beetles compared with 75 in the trap with the light and chemical. Only two beetles were captured in the chemically baited AE trap and one in the standard. Many other species of insects were caught, also.

MODIFICATIONS OF LIGHT-TRAPS.—Other investigators have used similar traps, both with and without baffles, for catching various insects. To determine whether baffles were essential, the three AE traps were spaced 40 feet apart and rotated every night for 6 nights, with each trap occuping the same position twice. A trap with the baffles removed caught 63 beetles, 1 with the baffles intact caught 74, and the third, with the baffles enclosed in a cylinder of 1-inch hexagonal weave poultry netting, caught 36. Three chemically baited traps in another row caught only five beetles during the 6 nights. No definite evidence was shown that the baffles were essential for maximum efficiency, but the poultry netting reduced the efficiency of the trap. The high efficiency of the blacklight was again demonstrated.

Further tests were made to determine whether a simpler and lighter trap could be designed without loss in efficiency. The AE trap required three standards arranged as a tripod. Four additional light-traps were constructed (fig. 1, right) by assembling the fluorescent lamp and the equipment required to operate it on a piece of $\frac{1}{8}'' \times \frac{3}{4}''$ flat iron. A funnel and receptacle similar to those on the plastic trap completed the unit. This made a light and compact trap which could be suspended from a single standard. Exclusive of the extension cord, one trap cost approximately $3.50. Although its over-all size was $11'' \times 30''$, it weighed less than 3 pounds. This unit is hereafter referred to as the Geneva trap.

During a 5-night exposure 1 Geneva trap captured 13 beetles, as compared with 18 in an AE trap in a similar environment. During a subsequent 28-night exposure a Geneva trap captured 42 beetles and an AE trap 26. Differences in location appeared to account for the differences in catches rather than differences in the traps. No beetles were captured in chemically baited traps during either period.

TIME OF NIGHT BEETLES ARE CAPTURED.—It was established during the first 2 nights of observation that light-traps attracted the beetles at some period other than at sunset. On July 25, when the traps were examined at 4 A.M., many of the beetles had already been caught, actually 66% of that nights' total. Too few beetles were in flight around these traps between 4 A.M. and dawn for satisfactory observation.

The traps were then examined at hourly intervals from 9 P.M. to 7 A.M. for 7 nights (July 30 to August 5). Peak flights occurred within 5 minutes of 9 P.M. during late June and early July and about 8:45 P.M. during late July and August. Beetles were captured during a 9-hour period throughout the night except from 1 to 2 A.M.. Most of these were captured during two periods—43 from 9 to 11 P.M. and 36 from 3 to 5 A.M. These figures are interpreted to indicate that most of the beetles flew to the trees for mating and thereafter were attracted to the lights. No beetles were captured by the chemically baited traps during these nights.

Earlier in the flight season (July 16 to 21) in connection with the testing of baited traps in another field, 2-watt argon ultraviolet lamps (General Electric AR-1), operating on d.c. from a portable generator, were exposed to beetles as they left the ground. Beetles were not attracted to these lights. After it was found that AE traps attracted beetles that had flown to trees, these lights were retested. One plastic trap was equipped with one and another with two argon lamps, and a third trap was equipped with a 15-watt incandescent lamp. These traps were operated throughout 6 nights, but none of them attracted beetles. Each of these traps and a chemically baited trap caught 1 beetle compared with 83 captured by an AE trap.

RELATIVE EFFICIENCY OF VISUAL OBSERVATIONS AND LIGHT-TRAPS.—For 12 nights the numbers of beetles flying around four trees surrounding the area where the traps were located were estimated and compared with the numbers captured in the light- and chemically baited traps. From three to seven traps of each kind were exposed. As indicated in table 1, the efficiency of the light-traps was high and tended to increase as the number of beetles in flight decreased. About one-third of all the beetles were captured. The light-traps caught 215 beetles as compared with only 3 in the baited traps.

EFFECT OF TRAP POSITION.—Since it was established that most of the beetles flew to the trees before they were attracted to the lights, it seemed evident that the proximity of the trap to the tree having the most beetles around it would influence its efficiency. In a given location the most beetles flew to the tallest tree.

Table 1.—Comparative efficiency of blacklight and chemically baited traps. Phelps, New York. 1958.

Night of Observation	Number of Beetles Around 4 Trees	Number of Beetles Captured	
		Light-traps	Baited Traps
July 22	200	41	2
24	200	73	1
27	80	31	0
30	80	13	0
31	70	5	0
Aug. 3	30	10	0
4	30	17	0
5	20	10	0
July 26	5	9	0
Aug. 11	0	1	0
13	0	4	0
15	0	1	0
Total	715	215	3

During the last of the flight season traps were placed at various distances from several trees. The most efficient trap was located under a large black walnut tree where beetle concentrations were the heaviest. From 50 to 90% of all the beetles around the four trees referred to in the previous experiment were in this tree. The trap under the tree caught 42 beetles from August 4 through September 2 as compared with 26 in a trap placed at the edge of the same tree. Not more than six beetles were caught by any other trap near smaller trees or in the open during the same period.

SEX RATIO OF BEETLES CAPTURED IN LIGHT-TRAPS.—The ratio of chafer males to females has been established as 2:1 (Gyrisco *et al.* 1954). At Geneva the ratio of beetles caught in chemically baited traps early in the season has been 10:1 or higher. The highest proportion of females has always been caught during the period of peak flight, at which time it has approached a 1:1 ratio; then the ratio of males has increased to the end of the season.

In chemically baited traps the ratio of males to females was similar to previous years' observations, but in the light-traps 137 males were captured to 220 females, for a ratio of 1:1.6.

DISCUSSION.—Although the AE trap was efficient in capturing beetles, its size and weight would make it unsuitable for most circumstances. Because of its smaller size and lighter weight the Geneva trap was an improvement, but additional modifications seem desirable. Red is the most attractive color for chemically baited traps, but the color of a light-trap is not believed to be a factor in its efficiency.

It has been established that chemically baited traps are most efficient when placed at a distance from tall trees (Tashiro & Fleming 1954); the opposite is true of light-traps. This fact partially overcomes the principal limitation of a light-trap in that isolated trees are frequently associated with dwellings where electricity is available to operate the trap.

Since the attractiveness of the 15-watt blacklight is not selective, the chafers must be sorted out from a large mass of other insects. However, this limitation could probably be reduced by regulating the size of the aperture into the collecting chamber and by providing a screened bottom, which would permit insects smaller than the chafer to escape.

Blacklight as an attractant to European chafer adults shows more promise than any chemically baited trap developed thus far and is a definite break-through in the search for a highly positive survey technique.

REFERENCES CITED

Gyrisco, G. G., W. H. Whitcomb, R. H. Burrage, C. Logothetis, and H. H. Schwardt. 1954. Biology of the European chafer, *Amphimallon majalis*, Razoumowsky (Scarabaeidae). Cornell Univ. Agric. Expt. Sta. Mem. **328:** 3–35.

Tashiro, H., and W. E. Fleming. 1954. A trap for European chafer surveys. Jour. Econ. Ent. **47**(4): 618–23.

Taylor, J. G., L. B. Altman, J. P. Hollingsworth, and J. M. Stanley. 1956. Electric insect traps for survey purposes. U. S. Dept. Agric., ARS **42–3,** 11 pp.

Estimating the Abundance of Corn Earworm Eggs[1]

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ABSTRACT

The moths of the corn earworm, *Heliothis zea* (Boddie), prefer to oviposit on fresh corn silk. A method is being sought, for use by field men, by which potentially heavily infested plantings can be located early enough for the successful application of insecticides. Two seasons' studies of the adaptation of black light traps to this use were unsuccessful. Wide variations existed between counts of eggs on corn silks made in the field and numbers actually present. A method was devised for removing eggs from silk for rapid counting under a low-power microscope. Silks were clipped from ears, brought to the laboratory and washed in clear gasoline in a 500 ml. flask. They were removed with a bristle-tipped stirring rod, the gasoline was floated and decanted off with 70% ethyl alcohol and the eggs, in the alcohol, were poured out into a watch glass.

The moths of the corn earworm, *Heliothis zea* (Boddie), lay eggs on some 70 weed and crop plants. However, they prefer to oviposit on fresh corn silk (Quaintance & Brues 1905). The number of eggs placed on the silks of any one plant is related to the number of moths in flight at the time, and to the number of attractive oviposition sites available (Phillips & Barber 1935, 1936). In Delaware these moths appear in early June (Milliron 1958) and increase in numbers as the season progresses. They frequently are abundant enough by the end of June to cause more or less noticeable infestation of any corn silking after that time. Also in this area several thousand acres of field corn are in silk between July 15 and August 5. With such an abundance of attractive egg-laying sites, the chances of oviposition on any single corn plant are lower then than during any other period after the moths appear. Delaware sweet corn processors take advantage of this fact and have the bulk of their acreage planted so that it silks in this favorable period. However, the moth population does not appear to be uniformly distributed. As a result more or less severe infestations occur every year in scattered fields. The processors would like a method of locating such susceptible plantings early enough to apply control measures.

METHODS.—For the past 4 years we have been investigating survey methods which might be used by field men, in the employ of sweet-corn processors, for locating earworm-infested fields early enough for the successful application of control measures.

Traplights.—In 1955 three traps equipped with ultraviolet lamps were operated in the vicinity of Massey, Maryland. One of these was operated daily at Massey from July 10 to August 25. The others were located near sweet-corn fields which silked in succession during this period, one being moved about at a distance of 4 to 6 miles from Massey, while the other was at a distance of 9 to 11 miles.

Relatively few moths were captured, and although the catch in all three traps usually rose and fell simultaneously, enough were taken for a good comparison during only one period (table 1). On nights when five or more moths were captured, the maximum deviation in the catch between the Massey trap and the other two, was 125% for the one located 4 to 6 miles distant and 389% for the one 9 to 11 miles away. These results suggested that, at best, such traps would have to be located less than 5 miles apart to provide an adequate index of moth activity for any given area.

In 1956 two of these traps were operated, one at

Table 1.—Corn earworm moth collections in blacklight traps. Vicinity of Massey, Maryland, 1955.

Date	Moths Captured			Per Cent Deviation from Massey Trap	
	At Massey	4–6 Miles from Massey	9–10 Miles from Massey	4–6 Mi.	9–11 Mi.
July 10		1	1		
11	2	1	1		
12					
13	1	3	1		
14					
15			1		
16			1		
17			1		
18		1	1		
19	1	1			
20	4	2			
21	1		1		
22	2	1	1		
23	1	1			
24	1	1	1		
25	2	2			
26	7	8	4	14	−43
27	4	6			
28					
29	3	1			
30	1		1		
Aug. 1	2	1	3		
2–7	No moths captured				
8			1		
9	2	1	11		
10	1	3	6		
11	3	4	8		
12	23	9	41	−61	78
13–14	severe storm—trap not operated				
15	9	19	44	111	389
16	13	23	21	77	62
17	7	15	13	114	86
18	8	18	19	125	137
19	8	11	7	37	−12
20	6	4	9	−33	50
21	4	5	3		
22	5	7	5	40	0
23	1	2	1		

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Table 2.—Corn earworm eggs found on 10 corn silks at each of seven locations by two methods of examination, Delaware, 1957.

Location	Type of Corn	Number of Eggs	
		Observed in Field	Found with Microscope
Fredrica	Field	0	4
Milford	Field	2	7
Ellendale	Field	11	12
Georgetown	Field	2	6
	Sweet	0	3
	Field	0	12
Bridgeville	Sweet	0	1

Georgetown and the other at Newark. During the entire period, July 5 to August 31, only 11 corn earworm moths were taken. The highest single night's catch in either trap was three. However, several of the plantings that were under observation near these traps developed corn earworm infestations. In one, silking August 1 and located 0.4 mile from the Georgetown trap, 70% of the ears were damaged. Thus it appeared that the black-light traps would not provide the early warning of infestation that was sought.

Field-Sampling.—In 1957 a study was begun to determine whether the probable degree of infestation could be estimated by sampling the corn earworm egg population of a field. For this a fairly simple and rapid method of counting the eggs was needed. To learn whether they could be counted with any degree of accuracy in the field, such counts were compared with microscopic examination of the silk. Ten fresh corn silks were examined in each of several fields for corn earworm eggs and the number found recorded. These silks were then clipped off and placed in labeled 1-pint plastic bags for return to the laboratory, where they were examined under a low-power microscope. The location of these collections together with the number of eggs found by the two methods is given in table 2.

It was apparent that, in most cases, numerous eggs were being overlooked in the field examination. Moreover, the number being missed varied widely from one field to another. The factors which appeared to have the greatest influence on the accuracy of the field count were the color of the silk, the visual acuity of the observer, the amount of moisture on the silk, the time of day, and the amount of light.

Microscopic examination of the silks, while it could be expected to give reproducible counts, is a tedious operation and for this reason appeared unsuitable as a technique for use by field men. Work was begun, therefore, and continued in 1958, on a method of removing the eggs so that they could more readily be counted.

The eggs of the corn earworm have been described by Quaintance & Brues (1905). They are white, dome-shaped, and have on the surface 28 ribs extending from near the apex toward the base. They are about 1/64 inch in diameter and appear to be glued in some manner to the silk (fig. 1). The silks, and the stigmatic hairs on their surface, are quite moist for some time after they protrude

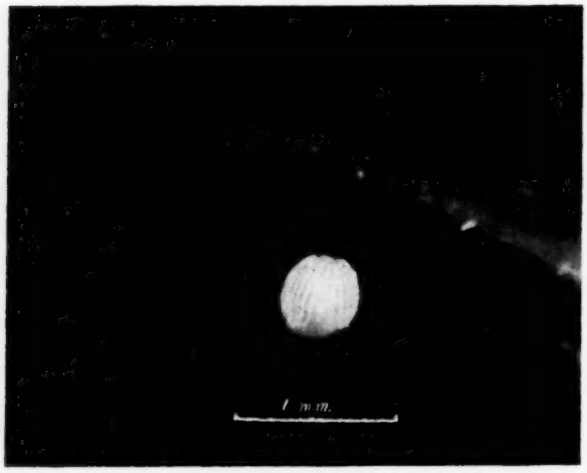

FIG. 1.—Egg of corn earworm on sweet corn silk.

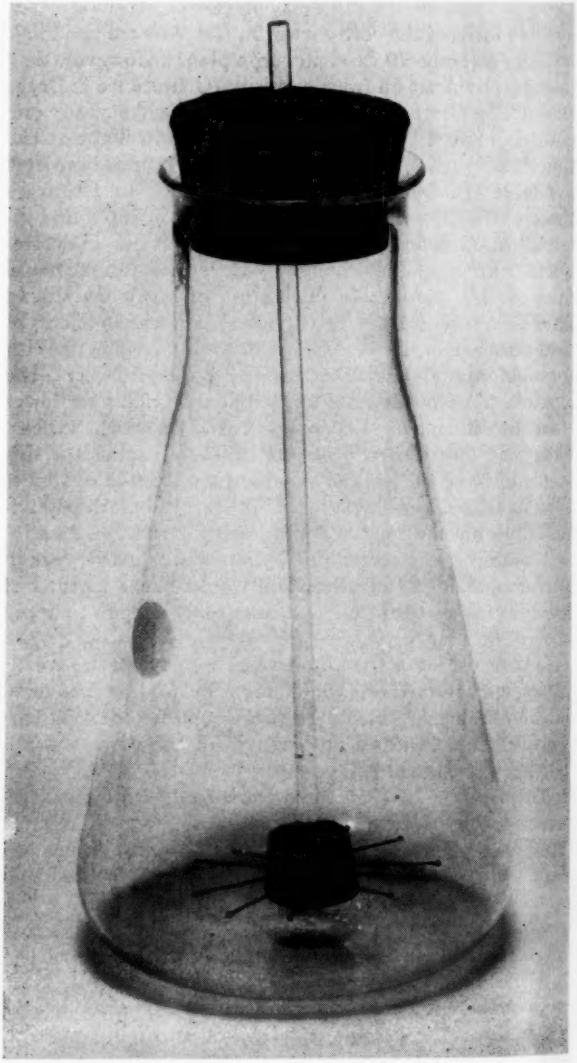

FIG. 2.—Apparatus for separating corn earworm eggs from corn silk.

from the husk. Should an egg become loosened from the spot where placed by the moth, it becomes trapped in this moisture and so remains on the silk.

Egg-Removal.—In experiments on removing the eggs, three to five silks bearing a known number of eggs were treated in stoppered 2-dram vials with some 25 different solvents and detergents. Of these, chloroform, xylene, and gasoline dislodged all the eggs within 1 to 3 minutes. Kerosene and ethyl acetate were less effective, requiring 15 minutes or longer to remove all of the eggs. The gasoline used was a clear, unleaded type. It is readily available and is the cheapest of the materials that proved effective.

Through further trials, the following method was developed for separating the eggs from quantities of silk. This is somewhat similar to that of the Association of Official Agricultural Chemists (1955) method for separating insect fragments from grain.

From 1 to 25 fresh silks, clipped from corn ears in the field and transported in a paraffin-coated paper cylinder (1-pint ice cream cartons), were placed in a 200 ml. wide-mouth flask (fig. 2). The flask contained a stirring rod with a No. 2 neoprene stopper at the tip. Eight 1/64-inch diameter spring-steel rods were anchored in this stopper in such a manner that they projected at right angles to the stirring rod and formed a circle of wire bristles just small enough to pass through the opening of the flask. To complete the assembly, another stopper was positioned on the rod so that it fitted snugly in the neck of the flask when the stopper bearing the bristles was in contact with the bottom. Sufficient gasoline was added to cover the silks. The stirring rod was centered in the flask and the upper stopper fitted into position. The silks were shaken with the gasoline for 3 minutes, after which they were drawn out of the flask by means of the bristle-tipped stirring rod and discarded. Some of the eggs dropped to the bottom while others, surrounded by a drop of water, stuck to the sides of the flask. Considerable debris remained suspended at various levels in the gasoline. Seventy per cent ethyl alcohol was then added. The gasoline and suspended material floated on the alcohol and was poured off. The eggs on the sides of the flask became detached and dropped to the bottom. More concentrated alcohol was not suitable as it tended to mix with the gasoline. Finally the eggs were poured out into a watch glass for counting.

REFERENCES CITED

Association of Official Agricultural Chemists. 1955. Methods of analysis. p. 75.

Milliron, H. E. 1958. Economic insect and allied pests of Delaware. Delaware Agric. Expt. Sta. Bull. **321**: 70.

Phillips, W. J., and G. W. Barber. 1935. The corn earworm as an enemy of field corn in the Eastern States. U. S. Dept. Agric. Farmers' Bull. **1651** (revised): 14–5.

Phillips, W. J., and G. W. Barber. 1936. Oviposition by *Heliothis obsoleta* Fab. on the silks of corn. Virginia Agric. Expt. Sta. Tech. Bull. **58**: 3.

Quaintance, A. L., and C. T. Brues. 1905. The cotton boll-worm. U. S. Dept. Agric. Bur. Ent. Bull. **50**: 1–155.

Dowco 109 as an Animal Systemic Insecticide[1]

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ABSTRACT

Because of its effectiveness in screening tests, DOWCO 109® (*O*-(4-*tert*-butyl-2-chlorophenyl)*O*-methyl methylphosphoramidothioate) was used to treat small numbers of cattle. The cattle grubs (*Hypoderma bovis* (L.) and *H. lineatum* (DeVill.)) were controlled by treating the animals orally or intramuscularly with 15 to 25 mg./kg. or by spraying them with a 1% suspension. The control varied from 76 to 99%. None of the animals were poisoned by the insecticide.

Although two new animal systemic insecticides, ronnel and Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) were recommended in 1958 by the Entomology Research Division (1958), laboratory screening and field testing programs are being continued at Kerrville, Texas. The objectives of these studies are to find new systemics that will be safer, more easily administered, and more effective against cattle grubs and other livestock pests.

One of the most promising of the new compounds is DOWCO 109® (*O*-(4-*tert*-butyl-3-chlorophenyl)*O*-methyl methylphosphoramidothioate). This insecticide was received at Kerrville in November 1956 after having shown promise in preliminary trials by research workers of the producing company. The results of our laboratory tests and early field trials are presented.

SCREENING TESTS.—Almost all candidate insecticides received at the Kerrville laboratory are routinely screened for systemic activity, following the procedures described by McGregor & Bushland (1956) and Drummond (1958). Oral dosages of 50 and 100 mg./kg. of DOWCO 109 administered to infested guinea pigs killed screw-worms (*Callitroga hominovorax* (Cqrl.)) and stable flies (*Stomoxys calcitrans* (L.)). The stable flies that fed 4 hours after treatment were killed but not those that fed at 24 hours. A dosage of 100 mg./kg. injected subcutaneously killed only the screw-worm larvae. Guinea pigs were reinfested with newly hatched larvae 24 hours after treatment. Lower oral and subcutaneous dosages were ineffective against screw-worms and stable flies. Attached nymphs of the lone star tick (*Amblyomma americanum* (L.)) and the guinea pig hosts were apparently unaffected by all dosages.

Because of the systemic insecticidal properties exhibited in the tests with guinea pigs, DOWCO 109 was tested against screw-worms in sheep. A wettable powder

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at 25% suspended in water was administered as a drench to two animals infested with 1- and 4-day-old larvae. A dosage of 50 mg./kg. killed larvae of both ages, but 20 mg./kg. was ineffective. R. D. Radeleff and G. T. Woodard, cooperating veterinarians of the Animal Disease and Parasite Research Division, observed that the sheep were not poisoned by the compound.

TESTS WITH CATTLE.—In the fall of 1956 two Hereford calves from a large group of cattle brought to Kerrville from Highmore, South Dakota, were treated with suspensions of DOWCO 109 made from a wettable powder at 10%. One animal was drenched at 10 mg./kg. and the other was sprayed with 2 liters of a 0.25% suspension. The treated animals and 16 untreated cattle were checked for encystment of grubs seven times from January through April 1957. An outline map was maintained for each animal to show the position of all grubs appearing in the back. In this way each individual grub was identified, and an accurate record of the total number in each animal could be kept.

No grubs appeared in the back of the orally treated animal, but 33 encysted in the sprayed one. Untreated animals had an average of 44.4 grubs per head.

Because of these promising results, additional tests were undertaken during 1957 with larger numbers of cattle from Texas and South Dakota. The Texas cattle consisted of 18 calves, which were approximately 6 months old and weighed 360–540 pounds, from the Government-owned herd on pasture at Camp Stanley. Eight of the animals were injected intramuscularly in the gluteal region with a solution containing 50% of DOWCO 109 furnished by the Dow Chemical Company, four at a dosage of 10 mg./kg. and four at 5 mg./kg. Eight animals were sprayed with a suspension of a wettable powder at 10%, four each with 0.5% and 1% concentrations. Each animal received 2 quarts applied with a power sprayer at a pressure of 100 pounds per square inch. The other two animals were fed DOWCO 109 in their ration at 1 and 0.5 mg./kg. daily for 42 days. Nine untreated animals were maintained as controls for these tests.

Manure from the animals in the feeding test was tested for toxicity to larvae of the horn fly (*Siphona irritans* (L.)) according to the technique of Eddy *et al.* (1954). Larvae survived in manure collected from both animals during and immediately following the treatment period.

Grubs started appearing in untreated animals about a month (August 15) after the cattle had been treated. All the cattle were examined at monthly intervals through January. Results of the examinations are summarized in table 1.

Neither of the injection treatments caused a significant reduction in grubs. Grubs appeared in only one of four animals sprayed with 1% DOWCO 109, and a highly significant reduction was indicated for the treatment. The reduction in animals sprayed with 0.5% was slight and not significant by the "t" test. The animal given DOWCO 109 in feed at 1 mg./kg. daily was infested with 35 grubs, but none appeared in the back of the animal treated at 0.5 mg./kg. The large number in the animal fed at the higher rate indicates that the treatment was ineffective, and no significance is attached to the failure of grubs to appear in the calf fed the lower rate.

The Hereford calves from South Dakota were 6 to 8 months old and weighed 230 to 415 pounds at the time they were moved to Kerrville. Twenty of these animals were treated with DOWCO 109 in November. Ten were injected in muscle of the gluteal area with a solution containing 50% of active ingredient furnished by the Dow Chemical Company, five each at 25 and 15 mg./kg. Five animals were drenched at 25 and five at 15 mg./kg. with a suspension made from a wettable powder at 25%. Twenty untreated animals served as controls. All animals were examined for warbles five times from January through April 1958. Results of the counts with these animals are included in table 1.

Table 1.—Cattle grub infestations in cattle treated with Dowco 109.

METHOD OF APPLICATION	DOSAGE	NO. ANIMALS		AVERAGE NO. OF GRUBS PER ANIMAL	PER CENT CONTROL
		In Test	Infested		
	Texas Cattle, Treated July 1957[a]				
Injection	10 mg./kg.	4	4	31	32
	5	4	4	42	7
Derma	1% 2 qt./head	4	1	9	80
	0.5%	4	4	34	24
Untreated	—	9	9	45	—
	South Dakota Cattle, Treated November 1957[b]				
Drench	25 mg./kg.	5	3[c]	2.0	94
	15	5	3	2.8	92
Injection	25	5[c]	2	0.5	99
	15	5	4	8.8	76
Untreated	—	20	20	36.4	—

[a] All grubs were *Hypoderma lineatum*.
[b] Both *H. bovis* and *H. lineatum*.
[c] One animal died from other causes.

Both drenches gave more than 90% reduction in grub emergence. The injection of 25 mg./kg. gave almost complete control but the lower dosage of 15 mg./kg. was significantly less effective.

The results of these tests show that DOWCO 109 is a highly active systemic insecticide against first-instar cattle grubs. It is effective when administered orally, intramuscularly, dermally, and possibly as a low-level feed additive. Unpublished results obtained by R. D. Radeleff indicated that small numbers of cattle treated orally with as much as 100 mg./kg. showed no symptoms of poisoning or serious blood cholinesterase depression. There appears to be a wide margin of safety between the dosage that is toxic to cattle and one that will give 90% or more control of cattle grubs, as none of the animals used in the tests showed any symptoms of poisoning.

REFERENCES CITED

Entomology Research Division. 1958. Insecticide recommendations of the Entomology Research Division for the control of insects attacking crops and livestock. U. S. Dept. Agric. Handbook 120, 110 pp.

Drummond, R. O. 1958. Laboratory screening tests of animal systemic insecticides. Jour. Econ. Ent. **51**(4): 425–7.

Eddy, G. W., W. S. McGregor, D. E. Hopkins, and J. M. Dreiss. 1954. Effects on some insects of the blood and manure of cattle fed certain chlorinated hydrocarbon insecticides. Jour. Econ. Ent. **47**(1): 35–8.

McGregor, W. S., and R. C. Bushland. 1956. Research on the use of systemic insecticides for the control of livestock pests. Jour. Econ. Ent. **49**(1): 86–8.

Apple Maggot Control Studies with Soil Insecticides[1]

E. R. Oatman[2]

Abstract

The effectiveness of several soil insecticides, applied to the sod in replicated plots, was evaluated for the control of the soil-inhabiting stages of the apple maggot, *Rhagoletis pomonella* (Walsh). The results of 1 year's study are reported. Endrin effected 96% reduction over the check. This reduction was significantly different from that given by dieldrin, aldrin, and heptachlor. The latter materials gave 66%, 63%, and 60% reduction, respectively. As endrin has already been accepted as an orchard rodenticide, its possibilities as a control measure for the apple maggot through soil applications is further enhanced. Its future, however, is contingent upon its residual control, a phase of research that is being continued.

The apple maggot, *Rhagoletis pomonella* (Walsh), is one of the most serious insect pests of apples in Wisconsin, principally in small isolated commercial orchards and in home orchards, where is is not uncommon to find 100% infestation of the harvested apples.

Control by foliar application is effected by one or more materials applied at 10 to 14 day intervals, beginning 7 to 10 days after the first adults emerge. Three or more sprays are normally required for adequate control. Thorough coverage and timing by this method are essential. Control in home orchards is especially difficult if there are neglected trees nearby.

The larva of the apple maggot emerges from the apple, enters the soil to pupate, and overwinters there. It is possible that a soil insecticide might be effective in controlling the insect in this phase of its life cycle or at the time the adult emerges from the puparium in the soil. Such insecticides have already been used successfully in the control of the plum curculio, which also pupates in the soil. Fluke & Dever (1954) reported that one application of aldrin and dieldrin, applied to the soil at 6 pounds actual per acre, gave 99% control of the plum curculio, *Conotrachelus nenuphar* (Hbst.), for 3 consecutive years. Snapp (1954) and Stelzer & Fluke (1958) also reported comparable control of this insect with similar applications of aldrin, dieldrin, and heptachlor.

Fluke & Dever (1954) included the apple maggot in their initial tests at Gays Mills in 1951–52. They reported that only dieldrin showed any apparent toxicity to the apple maggot and that control was not sufficient to warrant further studies. Cox (1955) reported that chlordane and dieldrin, used as soil insecticides in preliminary studies, gave only 56% and 67% control, respectively, against a closely related species, the cherry maggot, *Rhagoletis cingulata* (Loew).

According to C. L. Fluke (personal communication), the sod was removed before applying the insecticides (Fluke & Dever 1954). It was thought that this might have had an adverse effect on the control obtained for the apple maggot because it normally enters duff-covered sod to pupate. Dieldrin, however, did give partial control. Therefore it was decided to reevaluate some of the more promising soil insecticides for use as possible control measures when applied to the undisturbed sod. Endrin, which had not been included in previous tests, was included.

Table 1.—Evaluation of effectiveness of soil insecticides applied at 5.3 pounds of toxicant per acre for control of the apple maggot.

Emulsion Concentrate	No. of Adults Emerged	Per Cent Reduction	Treatment Mean	Analysis[a]
Endrin	34	95.8	8.50	a
Dieldrin	282	65.6	70.50	b
Aldrin	305	62.8	76.25	b
Heptachlor	327	60.1	81.75	b
Untreated	819	—	204.75	c

[a] Means designated by the same letter are not significantly different (5%). Based on Duncan's New Multiple Range Test—1955.

Methods and Materials.—The studies were established at the Peninsular Branch Experiment Station, Sturgeon Bay, Wisconsin, in 1957.

The insecticides used and rates of application are listed in table 1. Treatments were applied to 36-square-foot plots in a randomized complete block design, replicated four times. Equivalent amounts of insecticides were applied in 1 gallon of water using a 5-gallon knapsack sprayer. The sprayer was rinsed thoroughly after each treatment. The materials were further soaked into the sod by a 1-inch rainfall, which occurred 6 days after treatment.

The test site was located on undisturbed sod from which the grass was kept trimmed. A removable screen wire covered emergence cage, 2′×2′×12″, was fastened to its base with screen hooks. Each base, which was constructed of 1″×4″ pine, was sunk into the center of each plot to a depth of 2 inches. Each cage was stocked with 150 maggoty apples 1 month after treatment (fig. 1). A similar sample, held in funnels in the insectary, yielded 480 pupating larvae.

The adults were counted as they emerged during the summer of 1958. This count was accomplished by suspending a ¼″×8″×20″ piece of pressed fiber board in the center of each emergence cage (fig. 1). The boards were covered on two sides with Deadline, a sticky material used to trap the adult flies upon emergence. The adults were removed at 3- to 7-day intervals until cessation of emergence. This method for capturing the adults was very effective.

These studies will be continued to determine the residual toxicity of the materials to the various insects which spend a part of their life cycle in the soil.

Results and Conclusions.—The results of the 1957 soil insecticide treatments are shown in table 1. Endrin appeared especially promising as a means of control, being significantly better than aldrin, dieldrin, or heptachlor. Although the last three named materials pre-

[1] Project supported in part by research grants from the Shell Chemical Corporation and the Velsicol Corporation. Accepted for publication February 26, 1959.

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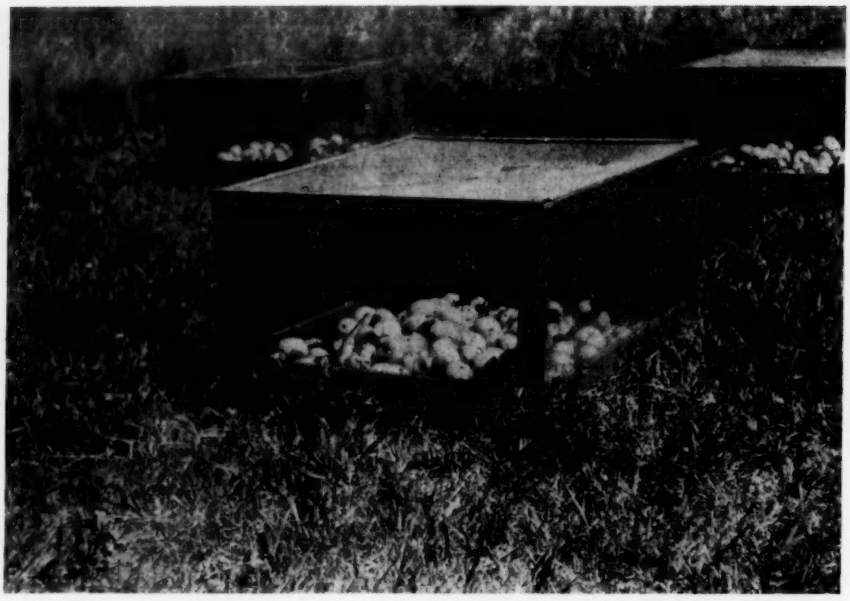

FIG. 1.—Emergence cages stocked with apple maggot infested apples. The Deadline—covered fiber board used for capturing the emerging adults is shown in place.

vented much less emergence of adults than the check, they did not give adequate control. The biotic potential of the adult maggot is such that near perfect control is necessary if the treatment is to be economically feasible.

The results indicate that the presence or absence of natural sod had no substantial effect on the toxicity of dieldrin to the apple maggot. The percentage of reduction over that of the check was only slightly higher than that obtained for dieldrin in the 1951–52 tests at Gays Mills by Fluke & Dever (1954). There was, however, a substantial increase in the percentage of reduction by aldrin, although Fluke & Dever (1954) found that it gave no reduction over the check plot.

Endrin at 5 pounds actual toxicant per acre also controls orchard mice. It also has distinct possibilities for the control of the plum curculio through soil applications. Should the one treatment continue to control the apple maggot, its application as a soil insecticide will provide a potentially effective and economical control of several of the most serious pests of apple production in Wisconsin. It would be especially applicable for use by home orchardists where the lack of costly power equipment for foliar application results in considerable loss from the apple maggot.

REFERENCES CITED

Cox, J. A. 1955. Insecticides for cherry fruit fly control. Jour. Econ. Ent. **48**(5): 575–6.

Fluke, C. L., and D. A. Dever. 1954. Soil application of insecticides to control plum curculio. Jour. Econ. Ent. **47**(6): 1115–7.

Snapp, O. I. 1954. Experiments on control of Georgia peach pests in 1953. Jour. Econ. Ent. **47**(5): 909–12.

Stelzer, L. R., and C. L. Fluke. 1958. Soil insecticides for plum curculio control in Wisconsin. Jour. Econ. Ent. **51**(2): 131–3.

NOTICE TO AUTHORS

Each manuscript submitted for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY must be accompanied by an abstract suitable for publication, and prepared in accordance with instructions for the preparation of abstracts as required by Biological Abstracts. This abstract will replace the usual author's summary. See "Important Notice to Authors" which appeared in the Bulletin of the Entomological Society of America 3(2): 1. June, 1957.

Authors are urged to consult the Publication Policies and Manuscript Rules and Suggestions (Vol. 49(4): 575–7) before preparing manuscripts intended for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY.

Feeding of Overwintered Tobacco Flea Beetles and First Brood Emergence Following Soil Treatment with Insecticides[1]

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Abstract

Injury by the tobacco flea beetle, *Epitrix hirtipennis* (Melsh.), is most noticeable on the foliage, but the larvae feed on the roots and may severely damage newly set tobacco.

Investigations were carried out during 1956–58, inclusive, to determine the effects of applying aldrin, chlordane, and heptachlor as a soil treatment on the feeding of the overwintered beetles and the emergence of the first brood adults.

A soil treatment with aldrin, chlordane, or heptachlor was less effective in preventing foliage damage to newly set tobacco than the standard DDT foliage spray applied in the plantbed before the plants were pulled. In the tests, the three insecticides gave similar results and no significant difference occurred in the aldrin or heptachlor treatments at 3 and 6 pounds per acre.

Aldrin, chlordane, and heptachlor were highly effective in preventing the emergence of the first brood adults on either an eroded Cecil loam or a Cecil sandy soil type. In the untreated plots a larger number of beetles emerged from caged plants on the eroded Cecil loam than from those on the Cecil sandy loam soil.

The tobacco flea beetle, *Epitrix hirtipennis* (Melsh.), is an important insect pest of flue-cured tobacco in Virginia. Injury by the adult to the foliage is most noticeable, but the larvae feed on the roots and may severely damage newly set plants.

During the past several years, broadcast soil treatment with insecticides has been investigated as a control for the tobacco wireworm, *Conoderus vespertinus* (F.), and other species of wireworms attacking newly set tobacco. Guthrie & Rabb (1956) reported excellent results with several chlorinated hydrocarbon insecticides applied broadcast. Aldrin, chlordane, dieldrin, or heptachlor, applied by this method, are now recommended practices for the control of wireworms attacking newly set tobacco.

The effect of insecticides applied by the broadcast method for control of the tobacco flea beetle on tobacco has received little attention. Dominick (1957) found that when certain insecticides were applied in transplant water with hand transplanters, a high reduction in the emergence of the first brood beetles from the soil around treated plants resulted. Hofmaster (1956) reported a high reduction in foliage and tuber damage by the spring brood potato flea beetle, *Epitrix cucumeris* (Harr.), as a result of broadcast soil treatment.

This paper reports the results of experiments conducted during the seasons of 1956–58, inclusive, to obtain information on the feeding of the overwintered beetles on newly set tobacco, and first brood emergence, following soil treatment with insecticides.

Procedure.—In 1956, the foliage feeding experiment consisted of 18 plots, each 16×26 feet; and in 1957, there were 15 plots, each 20×24 feet, at another location. All plots were separated by a 3-foot untreated area. Each treatment was replicated three times in randomized blocks. In the soil treatments, granulated insecticides were applied approximately 3 weeks before the plants were transplanted, and were immediately disked into the upper 2 inches of the soil. Tobacco plants set in all plots were relatively free of flea beetle feeding punctures on the transplanting date. In the DDT foliage-treated plots, the material was applied as a spray in the plantbed prior to pulling for transplanting to the field.

Data on leaf punctures were obtained by collecting the most injured leaf from each of 10 plants at random from the 2 center rows of each plot. Feeding punctures on the collected leaves were later counted in the laboratory and the average for each treatment computed.

In the emergence experiments, two soil types were used for the tests; a Cecil sandy loam and an eroded Cecil sandy loam soil. The treatments were replicated three times in a randomized block design at each location. The plots consisted of an area 12×12 feet, separated by a 3-foot untreated area on all sides. Soil treatment was accomplished with the use of granulated insecticides applied approximately 3 weeks before the transplanting date and immediately disked into the soil. Six untreated plants were set in a row prepared through the center of each plot.

The natural flea beetle population fed on the plants in the various plots for a period of 21 days after which all flea beetles were removed from the two center plants of each plot, and the plants individually caged and examined for newly emerged beetles in accordance with the procedure reported by Dominick (1957).

In 1958, a soil sample was taken from the stem and root area around two uncaged plants in each plot. Collected soil samples were washed through a series of screens for the recovery of the immature stages of the insect.

Statistical treatment of the results included the multiple range test for significance (Duncan 1955).

Results.—*Feeding.*—The insecticides used and foliar feeding punctures are presented in table 1. The three soil treatments, during both years, were less effective in preventing foliage damage to newly set tobacco than the DDT foliage treatment; however, they were superior to the check. No significant difference occurred in flea beetle feeding in plots treated with aldrin or heptachlor at 3 and 6 pounds per acre. Although a reduction in the amount of feeding occurred in the soil-treated plots, the control obtained was considered insufficient without a foliar treatment. This would be especially true during years when the overwintered adults are moderate to abundant.

Immature Stages.—On June 26, 1958, a soil sample was collected from the vicinity of the stem and root zone of two uncaged plants in each plot. With the exception of the chlordane treatment located on the eroded Cecil loam, no immature stages of the insect were found in

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Table 1.—Feeding of the overwintered tobacco flea beetle on newly set tobacco growing in soil treated with granulated insecticides as compared with foliage treatment.

Insecticide	Pounds of Active Ingredient per Acre	Average Number of Leaf Punctures[a,b]	
		1956	1957
Soil:			
Heptachlor	6	39.5	—
Aldrin	6	54.3	—
Heptachlor	3	54.6	26.0
Aldrin	3	53.0	22.7
Chlordane	4	—	35.1
Foliage:			
DDT 50% WP[c]	—	14.9	10.0
Check	—	92.3	51.1

[a] Average computed from 10 most injured leaves in each of three replicates. Leaves collected 14 days after transplanting.
[b] A break in the vertical line indicates 5% statistical significance between treatments.
[c] Two pounds per 100 gallons of water, applied at rate of 5 gallons per 100 square yards of plantbed area.

Table 2.—Emergence of first brood tobacco flea beetles from two types of soil treated with granulated aldrin, chlordane and heptachlor.

Insecticide	Pounds of Active Ingredient per Acre	Number of Flea Beetles Emerged[a,b]	
		1957	1958
	Eroded Cecil Sandy Loam		
Heptachlor	3	0.2	2.0
Aldrin	3	0.0	3.3
Chlordane	4	1.3	6.5
Check	—	21.7	41.2
	Cecil Sandy Loam		
Heptachlor	3	0.2	0.3
Aldrin	3	0.0	0.3
Chlordane	4	0.2	1.5
Check	—	10.5	17.7

[a] Average per plant.
[b] A break in the vertical line indicates 5% statistical significance between treatments.

samples taken from treated plots. An average of 0.2 immature tobacco flea beetle per plant was found in samples from the chlordane treatment. Samples from check plots showed an average of 3.2 pupae, prepupae and third instar larvae per plant on the eroded Cecil soil, and an average of 2.5 immatures of the insect in samples on the Cecil sandy loam. Plants removed from the check plots on the eroded Cecil soil were stunted in growth and showed root and stem damage.

Emergence.—The insecticides used, and first brood flea beetle emergence from soil around caged tobacco plants, are given in table 2. Either aldrin, chlordane, or heptachlor applied as a broadcast treatment gave highly effective and somewhat similar results in reducing the number of first brood flea beetles that emerged from the soil on both the eroded Cecil and sandy soil types. More beetles emerged from the soil around plants in the chlordane plots on both soil types, but this difference was not significant. In the untreated plots more than twice as many flea beetles emerged from the soil around caged tobacco plants located on the eroded Cecil sandy loam than from those on the Cecil sandy loam soil.

References Cited

Dominick, C. B. 1957. Insecticides in transplant water for prevention of tobacco flea beetle emergence. Jour. Econ. Ent. 50(3): 292–3.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 2(1): 1–42.

Guthrie, F. E., and R. L. Rabb. 1956. Broadcast treatment with insecticides and soil fumigation for tobacco wireworm control. Jour. Econ. Ent. 49(3): 344–7.

Hofmaster, R. N. 1956. Flea beetle control on Irish potatoes in Eastern Virginia. Jour. Econ. Ent. 49(4): 530–3

ERRATUM

In Vol. 52, No. 3, page 540, second column under "Results and Discussion," the fourth sentence should read as follows:

An analysis indicated that Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiate) and ethion were inferior to the other toxicants 1 day after treatment.

The sixth sentence should read as follows: Seven-day counts indicated that Diazinon, Dylox® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), ethion, and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) gave inferior control of thrips.

SCIENTIFIC NOTES

Field Control of *Drosophila* spp. in Cull Fruit[1]

HOWARD E. DORST, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

Drosophila flies, chiefly *Drosophila melanogaster* Meig., are troublesome pests to processors of tomato products. Contamination occurs in the field and processing plant when the flies deposit eggs in the cracks and crevices of the fruits. It has been suggested that the source of the flies in tomato fields in California is nearby peach orchards (Collins 1956, Michelbacher *et al.* 1954, Stombler *et al.* 1957). Peach orchards also appear to be the source in western Colorado and northern Utah. It is suspected that the flies move from the piles of cull fruit near the orchards to adjacent tomato fields which are harvested later.

In preliminary experiments conducted in 1949 and 1950, insecticide sprays on these piles gave poor control because they were applied only to the surface. Sodium benzoate, salicylic acid, and borax were also unsatisfactory. Slacked and unslacked lime was ineffective unless sufficient material was applied to smother the pile.

Since 1952 further experiments with insecticides have been conducted at Grand Junction, Colorado, to control drosophila breeding in piles of cull fruits. For these tests cull peaches or pears were placed in 10-bushel piles. These piles included overripe, green, blemished, undersize, and oversize fruit from the sorting sheds where peaches or pears were being graded for the market. Various insecticides, some at two strengths, were applied 2 to 4 days after the fruit was piled. In the early tests two or three applications of dust gave satisfactory control. In 1954 it was found that single applications of granules gave equally good control. Since then granules have been used at one-half pound per 10-bushel pile of fruit. From 4 to 9 replicates were included in each year's experiment. Control was determined by the number of flies that adhered to both sides of a 2-×36-inch fly ribbon laid over the center of the pile. The insects were activated by two strokes of the pyrethrum aerosol applied along each side of the ribbon. Counts were made 14 and 28 days after the granules were applied.

Table 1.—Drosophila control in piles of cull fruit with single applications of granulated insecticides, 1954–58.

Insecticides	Years in Test	Per Cent Control After—	
		14 Days	28 Days
Endrin 2.5%	1	92	99
Lindane 5%	2	99	98
Malathion 15%	1	96	97
Diazinon 2.5%	2	96	95
BHC 5%	3	98	94
Diazinon 1%	1	97	92
Dieldrin 5%	4	96	92
Heptachlor 5%	3	88	91
Lindane 2.5%	1	98	91
Aldrin 2.5%	3	96	90
Heptachlor 10%	1	90	89
Dibrom® 5%[a]	1	97	89
DDT 10%	1	93	87
Aldrin 5%	1	90	86
DDVP 5%	1	98	84
Dieldrin 2.5%	1	94	84
Chlordane 10%	2	92	83
Parathion 5%	1	90	80
Malathion 10%	5	97	72
Chlordane 5%	3	95	78
Thimet® 2%[b]	1	96	66
DDT 2.5%	1	81	63
Heptachlor 2.5%	2	88	57
Disyston® 5%[c]	1	92	56
Ronnel 5%[d]	1	88	56
Guthion® 5%[e]	1	65	56

[a] 1,2-Dibromo-2,2-dichloroethyl dimethylphosphate.
[b] *O,O*-diethyl *S*-(ethylthio) methyl phosphorodithioate.
[c] *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate (also designated Bayer 19,639).
[d] *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate (also designated Dow ET-57, Korlan, or Trolene).
[e] *O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate.

The population of drosophila in piles of cull fruit varied from year to year. Table 1 gives the control obtained in tests with single applications of 17 granulated insecticides which have been arranged according to their efficiency in reducing the number of flies 28 days after treatment. Four materials gave sustained reduction of flies over this period of 95% or more. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiate) at 2.5% strength was among the four best; it was almost as effective at 1% strength.

References Cited

Collins, W. E. 1956. On the biology and control of *Drosophila* on tomatoes for processing. Jour. Econ. Ent. 49(5): 607–10.

Michelbacher, A. E., and Middlekauff, W. W. 1954. Vinegar fly investigation in northern California. Jour. Econ. Ent. 47(5): 917–22.

Stombler, Victor, C. D. Pelekassis, and Edwin S. Doyle. 1957. *Drosophila* control on harvested tomatoes for processing in California. Jour. Econ. Ent. 50(4): 476–80.

[1] In cooperation with the Utah and Colorado Agricultural Experiment Stations. Accepted for publication February 3, 1959.

Some Observations on the Development of the Boll Weevil on the Wine Cup, *Callirrhoe involucrata* (Nutt.) A. Gray[1]

J. K. WALKER, JR., *Texas Agricultural Experiment Station, College Station*

In the United States, two plants other than the cottom plant have been observed to serve as hosts for the boll weevil, *Anthonomus grandis* Boh. These are *Hibiscus syriacus* L. and *Thespesia populnea* (L.) Soland (Gaines 1934) and (Lukefahr 1956). Both plants are members of the family Malvaceae.

In Central Texas a very common malvaceous plant, the wine cup, *Callirrhoe involucrata* (Nutt.) A. Gray, blooms and matures during April and May. This mallow can serve as host for *Anthonomus fulvus* LeConte (Mitchell & Pierce 1911). Coad (1913) noted that overwintered boll weevils would feed on and oviposit in the wine cup. This worker implanted eggs of the boll weevil into four wine cup buds. No adult weevils emerged from these buds although a later examination indicated that larval feeding had occurred in one bud. To investigate the further possibility of the boll weevil developing in the wine cup, work was conducted at the Entomology Research Laboratory at College Station, Texas.

In April 1957, boll weevils were removed from hibernation cages and transferred to small screen cages in the laboratory where they were provided with buds of the wine cup for food and

[1] Technical contribution No. 3189, Texas Agricultural Experiment Station in cooperation with Entomology Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Accepted for publication March 16, 1959.

oviposition. After a period of 2 weeks examinations of the buds revealed the presence of boll weevil eggs. The insects also oviposited within the maturing fruit called capsules. In several instances, eggs were found deposited on the outside surface of the buds and capsules.

Boll weevil eggs were removed from cotton squares and implanted individually in wine cup buds. Each bud was partially enclosed with a thin layer of paraffin to prevent premature opening. Out of 40 buds in which eggs were implanted, seven adult weevils emerged. Examination of other previously implanted buds indicated that a large percentage of the eggs hatched and the larvae lived for several days. There was a high incidence of fungus contamination in buds in which eggs were placed. This may account for the low yield of adults. Six capsules of the wine cup were implanted with eggs but no adult weevils emerged. Observations of other capsules implanted with weevil eggs indicated that little or no larval development occurred.

In April 1958 newly emerged pairs of boll weevils were enclosed with wine cup buds as their only source of food. Copulation was first noted 6 days after confinement and the weevils commenced to deposit eggs in the buds approximately 7 to 9 days after emergence. Four of the eggs deposited by these weevils were removed from buds, placed on moist filter paper and held at 80°F. All hatched within a 3-day period.

Owing to the more rapid development of the wine cup bud in comparison with the cotton square, a weevil would be forced to oviposit in a very small bud of a growing wine cup in order to allow sufficient time for the attainment of complete growth of the larva.

REFERENCES CITED

Coad, B. R. 1913. Feeding habits of the boll weevil on plants other than cotton. Jour. Agric. Res. **2**(3): 235–45.

Gaines, R. C. 1934. The development of the boll weevil on plants other than cotton. Jour. Econ. Ent. **27**(4): 745–8.

Lukefahr, M. 1956. A new host of the boll weevil. Jour. Econ. Ent. **49**(6): 877–8.

Mitchell, J. D., and W. Dwight Pierce. 1911. The weevils of Victoria County, Texas. Proc. Ent. Soc. Washington, **13**: 45–62.

Confirmation by Culture and Staining Methods of Absence of Symbiotic Flora in *Tribolium confusum* Duval[1,2]

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Considerable experimentation has been done on the nutrition of the confused flour beetle, *Tribolium confusum* Duval, on the apparent assumption that no symbiotic flora was present in the digestive tract. Steinhaus (1946) mentions the observations of Chapman (1924), who states that the digestive tract of *T. confusum* larvae is sterile. Chapman's conclusion was based solely upon microscopic examination of stained intestinal contents of several larvae; the stain employed was not specified. No attempt to culture intestinal contents of larvae or adults was reported.

Steinhaus also refers to papers by Pierantoni (1929) and Koch (1930). Pierantoni reported the absence of intracellular symbiotes in *T. confusum;* whereas Koch demonstrated, by means of sections stained with Altmann's acid fuchsin and methyl green, and in a few cases with Heidenhain's haematoxylin, the presence of slender, rodlike organisms in mycetocytes throughout the fat bodies. In both these papers only staining techniques were reported; no culture methods were used.

In connection with nutritional studies currently being done with adults of *T. confusum* in our laboratory, the authors desired, primarily, to test the validity of Chapman's conclusion, and secondarily, to check the tissues for the possible presence of intracellular symbiotes. Two approaches were utilized. An attempt was made to culture any bacterial symbiotes which might be present in the digestive tract. In addition, the whole abdominal contents were dissected from the exoskeleton, were fixed, sectioned and then stained with bacterial and other stains in an effort to localize intra- and extra-cellular symbiotes.

All but two of the beetles used for this study were taken at random from a stock culture maintained on a nonsterile mixture of rolled oats, fish meal and dry brewer's yeast. Under laboratory conditions this culture medium was found to be contaminated by micro-organisms. A small sample of the food mixture, which had been in the stock culture for approximately 1 month, and a small sample of freshly mixed food were each transferred separately into two bottles of nutrient broth and incubated at 37° C. for 24 hours. All four of the bottles of broth became cloudy, and a surface growth of mold was noted on each. Nutrient agar platings from each broth bottle yielded gram-positive, spore-forming rods. The other two beetles which were used had been fed on a pure chemical diet, and tissues from both of these were included only in those sections stained with methylene blue.

Three inoculation experiments were conducted, using a total of 24 beetles; an additional 29 were used for histological sections. For the inoculations, beetles were dissected under sterile conditions and the intestinal tract from each beetle was cut into small segments. Some pieces were put into individual bottles containing about 70 ml. of sterile insect saline, and other pieces from the same beetle were transferred directly into tubes of nutrient broth. Most of the broth tubes were incubated at 32° C; a few were incubated at 37° C. From each saline bottle containing intestinal segments, inoculations were made into tubes of each of four different media (nutrient agar, chocolate agar, dextrose agar, and North's gelatin agar). Thus, samples from each beetle were inoculated into five different kinds of bacterial media. The agar tubes were incubated at the same temperatures as the broth. In the first experiment, in which 12 beetles were used, all inoculated media were incubated under aerobic conditions. The other two experiments utilized six beetles each but, in these cases, two tubes of each medium were inoculated from each beetle, with one set incubated under aerobic conditions, and the other set from each beetle incubated under anaerobic conditions. Oxygen was evacuated from the anaerobe tubes by means of pyrogallol and sodium hydroxide. After a period of several days, when it was reasonably certain that no bacteria were present in the anaerobe tubes, the oxygen content of several of these tubes was determined with a Beckman model D oxygen analyzer and found to vary from 0.5 to 1.5%. Except for a few presumed contaminants, irregularly distributed among the 180 incubated tubes, results were negative in all three experiments.

Guts from 29 beetles were sectioned. Sections from 24 were stained with Gram-Weigert stain for the detection of bacteria in tissues, and sections from five with methylene blue for the same purpose. Staining procedures outlined by Guyer (1953) were followed. In addition to the above stains, Mayer's haemalum, toluidine blue, mercuric bromphenol blue, the Feulgen technique (acid fuchsin and alcoholic fast green), methyl green-pyronin, and azure blue have been used as a routine part of the authors' nutritional studies. No micro-organisms were observed on any of the slides, either within the tissues or within the lumen of the various parts of the digestive system.

CONCLUSION.—Adults of *Tribolium confusum* Duval taken from the stock culture in our laboratory appeared to be free of intestinal bacteria when tested by both aerobic and anaerobic culture methods, and showed no evidence of tissue infections

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when sections were treated with bacterial and other stains. An explanation for the discrepancy between Koch's results and those reported here regarding intracellular symbiotes is the possibility that symbiotes may be peculiar to *T. confusum* under certain cultural conditions or in some geographic locations.

REFERENCES CITED

Chapman, Royal N. 1924. Nutritional studies on the confused flour beetle, *Tribolium confusum* Duval. Jour. Gen. Physiol. **6**: 565–85.

Guyer, Michael F. 1953. Animal Micrology. Chicago, Ill., The University of Chicago Press.

Koch, Anton. 1930. Über das Vorkommen von Mitochondrien in Mycetocyten. Zeitschr. Morphol. u Ökol. Tiere **19**: 259–90.

Pierantoni, U. 1929. L'organo simbiotico di *Silvanus surinamensis* (L). Atti Accad. Naz. Lincei. Rend. Cl. Sci. Fis. Mat. e Nat. 9(6): 451–65.

Steinhaus, Edward A. 1946. Insect Microbiology. Ithaca, New York, Comstock Publishing Company, Inc.

Comparative Yields of Cotton in Treated and Untreated Plots in Insect-Control Experiments in Central Texas, 1939-1958[1]

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Losses to the cotton crop caused by insects are of considerable concern to those interested in cotton production. It is difficult to determine the severity of these losses over large areas. Research data are available in some localities which may serve as a basis for estimating, with certain limitations, the amount of damage that insects have caused to the crop over a period of years. It is the purpose of this paper to present such data obtained during the 20-year period 1939–58, in central Texas.

Field experiments for the control of cotton insects have been conducted each year since 1939, when the U. S. Department of Agriculture Cotton Insects Laboratory was established at Waco. The primary objective has been to evaluate insecticides and methods of application against the various pests. Yields from plots or fields treated with candidate insecticides have been compared with those receiving a standard insecticide and with those receiving no treatment. These experiments were conducted under varied conditions, principally in McLennan and Falls Counties, and in some years also in Limestone and Milam Counties. These comparisons may, therefore, serve as a basis for estimating crop losses to insects in fields receiving no insecticides. However, it is realized that the use of the best insecticides does not prevent all insect damage and that a certain amount of damage can be tolerated before the use of an insecticide is economically feasible. The development of more efficient insecticides, methods of application, and control programs over the years has resulted in better insect control and usually in greater increases in yield. No attempt is made to evaluate insect damage to grade and staple of lint, because such data are available for only a few years.

Data for the 20-year period are presented in table 1.

Small-plot experiments with replicated treatments, and large-scale experiments with treatments on a large-plot, field, or community-wide basis were used in the study. The areas in the small-plot experiments ranged in size from 0.05 to 0.2 acre, those in large-plot and field experiments from 2 to 20 acres, and those in community-wide experiments from several hundred to several thousand acres.

The insects of primary importance in most years were the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis zea* (Boddie)). The cotton fleahopper (*Psallus seriatus* (Reut.)) was involved in many years, and the cotton aphid (*Aphis gossypii* Glov.) was of considerable importance until after the mid-1940's when several chlorinated hydrocarbon insecticides other than DDT were available. Infestations of the cotton leafworm (*Alabama argillacea* (Hbn.)) and the desert spider mite (*Tetra-*

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[2] In Cooperation with the Texas Agricultural Experiment Station.

Table 1.—Number of plots, acres and insecticide applications, and yields in experiments conducted in central Texas, 1939 to 1958.

YEAR	NO. OF EXPERIMENTS	NO. OF PLOTS		NO. OF ACRES		AVERAGE NO. OF APPLICATIONS	POUNDS OF SEED COTTON PER ACRE		GAIN OVER UNTREATED (%)
		Untreated	Treated	Untreated	Treated		Untreated	Treated	
1939	10	44	56	16	28	4.6	615	676	9.9
1940	10	47	47	14	49	5.2	767	922	20.2
1941	14	52	52	15	15	7.0	523	1088	108.0
1942	18	51	48	18	25	5.7	874	1039	18.9
1943	11	35	35	10	13	5.1	1166	1307	12.1
1944	8	29	29	10	10	4.6	831	1095	31.8
1945	11	29	32	11	60	5.3	941	1311	39.3
1946	15	36	36	28	43	5.9	403	1034	156.6
1947	18	34	44	50	111	4.5	699	907	29.8
1948	21	55	89	57	231	4.9	483	830	71.8
1949	17	26	35	485	597	5.4	828	1350	63.0
1950	20	57	83	1089	3826	4.7	1036	1357	31.0
1951	21	37	50	64	145	4.1	539	697	29.3
1952	28	50	93	76	117	4.7	560	810	44.6
1953	26	44	146	80	333	4.5	791	1054	33.2
1954	18	31	111	69	216	2.9	446	578	29.6
1955	11	29	53	21	51	6.4	778	1461	87.8
1956	17	45	48	28	37	5.1	830	1122	35.2
1957	16	34	191	27	140	7.3	1012	1270	25.5
1958	10	22	107	9	55	7.0	673	1070	59.0
Totals	320	787	1385	2177	6102				
Average						5.2	740	1049	41.8

nychus desertorum Banks) usually were not permitted to develop to proportions that might influence results against the test insect.

During the first 10 years few experiments were conducted for the control of early-season insects, because there were no materials that would control combinations of several pests and when these did become available the dust method of application then in use did not produce satisfactory results in this area. Beginning with 1949, when the low-volume spray method of applying the chlorinated hydrocarbon insecticides was developed, a combination of several species including thrips (*Frankliniella* sp.), cotton fleahoppers, and overwintered boll weevils were involved each year in experiments conducted for the control of early-season insects.

Insecticides were applied principally as dusts from 1939 to 1948, although high-volume sprays were sometimes used in 1939 and 1944 and low-volume sprays in 1945 and 1948. Thereafter, insecticides were applied as low-volume, low-pressure emulsion sprays although dusts were included each year in some experiments for comparative purposes. The experiments were conducted under natural moisture conditions except in the last 3 years, when a few of the late-season experiments were conducted in fields receiving irrigation.

Areas treated with standard insecticides and with candidate insecticides that approached or surpassed the standard in cotton production were included in the yield comparisons. An average of yields from the various experiments was used in computing annual yields.

Substantial increases in yield were obtained each year except in 1939 and 1943. The average increase for the period was 309 pounds of seed cotton per acre or 42%. The largest increases occurred in 1941, 1946, and 1955 when insect damage was greatest. Increases exceeded 50% in 6 years and 25% in 16 years of the period. Prior to 1945 when inorganic insecticides such as arsenicals and sulfur were relied on for insect control, the average increase in yield was 34%, but since the advent of organic insecticides it has been 53%.

The data clearly show the value of cotton-insect control with insecticides and also serves as an estimate of insect losses suffered in untreated cotton fields during the 20-year period in the central Texas area.

Field Insecticide Tests for Alfalfa Weevil and Pea Aphid

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ABSTRACT

Seven insecticides, including two new systemics, were tested against the alfalfa weevil, *Hypera postica* (Gyll.), and the pea aphid, *Macrosiphum pisi* (Harris), in randomized, replicated field plots. Materials used and rates of application are included. April 30 was the spray date. Results were obtained by sampling insects with an insect net, May 9, 16, and 29, The percentage of control, or reduction of infestation, was obtained for each material for each date.

The new systemics, American Cyanamid Company Compound 12880 (*O,O*-dimethyl-*S*-(N-methylocarbamoylmethyl) phosphorodithioate) and Compound 18706 (*O,O*-dimethyl-*S*-(N-ethylcarbamoylmethyl) phosphorodithioate), showed promising results against the pea aphid (97% reduction in numbers 16 days after treatment). These chemicals gave a quick reduction of alfalfa weevil adults (98% and 96% control at the 9-day sampling), and Compound 12880 substantially reduced the weevil larvae for the same period.

All materials except Compound 18706 produced some chlorosis in the alfalfa; however, Compound 12880 produced only a negligible amount.

Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3,-benzotriazine-3-methyl) phosphorodithioate) performed well against both pea aphid (89% control at the 16-day sampling) and the alfalfa weevil, at 9 days.

None of the test materials at the dosages used appeared as effective against alfalfa weevil as heptachlor combined with malathion.

In the spring of 1958 seven relatively new insecticides, including two promising systemics, were tested against insects on alfalfa. These insecticides were part of a continuing search for a completely safe, effective, and economical control for the important pests. Data were taken on the alfalfa weevil, *Hypera postica* (Gyll.), and the pea aphid, *Macrosiphum pisi* (Harris).

PROCEDURE.—The tests were conducted on plots 30 feet wide by 100 to 150 feet long in randomized blocks, replicated three times, in a field near Waterloo, Maryland. The materials were applied with a tractor-drawn sprayer carrying a 30-foot boom equipped with flat-spray nozzles. All chemicals were applied in 25 gallons of water per acre at 100 pounds pressure per square inch. The alfalfa was approximately 10 inches tall on the date of treatment, April 30.

The insecticides tested, with pertinent data, are listed in table 1. Dibrom® (*O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate), Dylox® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), Guthion® (*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate), Sevin® (1-naphthyl-*N*-methyl carbamate), and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) are established in the literature. Included in the tests were two entirely new systemics furnished by the American Cyanamid Company—Compound 12880 (*O,O*-dimethyl *S*-(*N*-methylocarbamoylmethol) phosphorodithioate) and Compound 18706 (*O,O*-dimethyl *S*-(N-ethylcarbamoylmethyl) phosphorodithioate.

Prespray and postspray population estimates were made by sweeping with a 15-inch sweep net. Population estimates on April 30, May 9, and May 16 are based on 10 sweeps taken from two points on each plot. Because of the large population of alfalfa weevil larvae, only one or two sweeps per plot were made on May 29. The prespray estimates are given to indicate the degree of variability of insects over the field. All estimates are reduced to the number of insects per sweep. Comparisons are made between each treatment and the untreated check plot, sampled the same day, "per cent control" being the percentage of reduction in infestation expressed to the nearest whole number.

Comparisons were also made between the experimental plots and a nearby field which was sprayed May 2 with a mixture of 6 ounces actual heptachlor and 15 ounces actual malathion in 25 gallons of water per acre, the standard Maryland recommendation, using the same spray machine as was used in the experi-

Table 1.—Insecticides tested against alfalfa insects, Maryland, 1958.

MATERIAL	MANUFACTURER	CONTENT (LB./GAL.)	POUNDS/ ACRE ACTUAL
Dibrom	California Spray-Chemical Corp.	8	1
Dylox	Chemagro Corp. (Bayer)	50% W.P.	1
Guthion	Chemagro Corp.	1.5	1
Sevin	Union Carbide Chemicals Co.	1	1
Thiodan	Niagara Chemical Division	2	0.5
Compound 12880	American Cyanamid Co.	4	1
Compound 18706	American Cyanamid Co.	2	1

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Table 2.—Effectiveness of various insecticides tested against alfalfa weevil and pea aphid on alfalfa, showing number of insects per sweep and degree of control,[a] on successive dates.

	Treatments					Am. Cyanamid		Check
	Dibrom	Dylox	Guthion	Sevin	Thiodan	12880	18706	
				Alfalfa weevil adults				
April 30	0.77	0.83	0.60	0.90	1.13	0.73	0.37	0.80
May 9	0.57 (66)	0.33 (80)	0.07 (96)	1.03 (38)	0.50 (70)	0.03 (98)	0.07 (96)	1.67
May 16	0.30 (19)	0.33 (11)	0.27 (27)	0.50	0.83	0.33 (11)	0.43	0.37
				Alfalfa weevil larvae				
April 30	1.20	0.80	0.97	2.53	1.20	1.03	0.67	0.53
May 9	14.4	11.5	0.6 (94)	6.1 (44)	2.2 (80)	0.8 (92)	3.1 (72)	10.9
May 16	27.8 (15)	26.5 (20)	5.3 (84)	15.9 (51)	5.3 (84)	7.8 (76)	22.6 (31)	32.7
				Pea aphids				
April 30	7.1	2.4	1.7	7.4	5.9	8.0	1.3	4.2
May 9	1.6 (87)	9.7 (19)	0.1 (99)	8.7 (28)	0.4 (97)	0 (100)	0.03 (99)	12.1
May 16	6.6 (68)	20.5 (0.5)	2.2 (89)	26.3	2.6 (87)	0.6 (97)	0.5 (97)	20.6

[a] Per cent control given in parentheses.

ment. Sweeps were taken May 16 and again May 29.

RESULTS AND DISCUSSION.—Results are shown in table 2, first considering adult alfalfa weevils. At the first postspray examination, May 9, Guthion and Compounds 12880 and 18706 gave reductions (per cent control) of adult weevils of 96% or more. Dylox, Thiodan, Dibrom, and Sevin gave reductions of 80, 70, 66, and 38%, respectively. On May 16, control from the various insecticides had fallen to 27% or less.

In the standard sprayed field 0.1 adult weevils were found per sweep on May 16, an indicated control of 73 %.

Against alfalfa weevil larvae on May 9, Guthion and Compound 12880 gave reductions of more than 90%, Thiodan 80%, and Compound 18706 gave 72%. The remaining materials were not effective. On May 16, control of larvae was relatively poor, Guthion and Thiodan leading with 83% and Compound 12880 next best with 76%. Control of weevil larvae in the standard sprayed field on May 16 was perfect, with no larvae taken in 20 sweeps. By May 29, weevil larvae had increased greatly on all plots, with infestations ranging from 35 to 129 per sweep. The standard sprayed field showed a similarly high infestation of 74 weevil larvae per sweep.

Against pea aphids on May 9, four insecticides showed 96% control or better—Compounds 12880 and 18706, Guthion, and Thiodan. Dibrom gave a fair reduction, but Dylox and Sevin were not effective. The May 16 results were also very good for Compounds 12880 and 18706 and fair for Guthion and Thiodan. The population of pea aphids in the standard sprayed field on May 16 was 8.1 per sweep, an indicated control of 33%.

Spray Injury.—On May 9 considerable injury was apparent to the alfalfa from several materials. The uppermost leaves showed a reduction of chlorophyll, or blanching, and some withering. An estimate was made of the percentage of plants affected at two points in each plot, or at six points for each treatment. The estimates of injury averaged: Sevin 80%; Dibrom 73%; Guthion 52%; Thiodan 50%; Dylox 41%; Compound 12880 showed 3%, and Compound 18706 showed none. Most of the injury on all the plots disappeared by the end of the month, and apparently no plants were killed. Safety in the systemics was marked.

Residue Analysis.—At the request of the Niagara Chemical Division, samples of alfalfa were taken from the Thiodan-treated plots for residue analysis. These samples were taken on May 27, 27 days after treatment, when the alfalfa was ready to be harvested. The report showed an average of 0.09 p.p.m. Thiodan on treated samples.

CONCLUSIONS.—The two systemics Compounds 12880 and 18706 showed promising results against the pea aphid, giving 97% reduction in numbers 16 days after treatment. These chemicals gave a quick reduction of alfalfa weevil adults (98% and 96% control at the 9-day sampling), and Compound 12880 substantially reduced the weevil larvae for the same period.

All materials except Compound 18706 produced some chlorosis in the alfalfa; however, Compound 12880 produced only a negligible amount.

Guthion performed well against both pea aphid (89% control at the 16-day sampling) and the alfalfa weevil at 9 days. None of the test materials at the dosages used appeared so effective against alfalfa weevil as heptachlor combined with malathion.

The Effects of Certain Insecticides on the Chinch Bug on Grain Sorghum[1]

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The chinch bug, *Blissus leucopterus* (Say), began to appear in damaging numbers on grain sorghum and corn in northeast Texas during the latter part of June 1958. Apparently the insects migrated by flight from fields of small grain that contained light infestations earlier in the growing season. Nymphs did not crawl from the small-grain fields to grain sorghum, as occurs in some sections of the United States.

MATERIALS AND METHODS.—An experiment was conducted in Kaufman County in 1958 to evaluate the effectiveness of toxaphene, methyl parathion, aldrin, malathion, heptachlor, Trithion® (*S*-*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2-4-3-benzo-dioxathiepin-3-oxide), and dieldrin for the control of the chinch bug on grain sorghum. The sprays were applied with a self-propelled machine equipped with No. 3 hollow-cone nozzles attached directly to a horizontal boom and spaced at 40-inch intervals. Sprays, prepared from emulsifiable concentrates, were applied at the rate of 4 gallons per acre. Two applications of sprays were made. The grain sorghum plants were 14 to 18 inches high at the time of the first application. This method of applying the insecticides did not prove satisfactory because the sprays were not directed to lower portions of the plants where the insects fed. The second treatment was made when the plants were from 20 to 30 inches high. Two nozzles were attached to a swivel at the lower end of 18-inch drops spaced at 40-inch intervals along the horizontal spray boom. By adjustment of the swivels the spray material was applied to the lower portions of the plants where the chinch bugs congregated.

[1] Contribution No. 3166, Texas Agricultural Experiment Station. Accepted for publication February 13, 1959.

Table 1.—Results of insecticide treatments for the control of the chinch bug on grain sorghum in Kaufman County, Texas. 1958.

Treatment[a]	Toxicant (Lb./A.)	Chinch Bugs per 5 Plants on July 2	Per Cent Control After Treatments[b] July 7	July 12	July 18	July 25
Toxaphene	3.00	82.8	89.9	86.1	93.6	87.3
Methyl parathion	.25	138.5	66.0	46.1	67.8	0
Aldrin	.50	84.5	67.5	26.3	68.9	37.5
Malathion	.75	88.5	1.8	0		
Heptachlor	.50	64.0	12.5	23.9	57.5	0
Trithion	.50	62.3	1.2	5.5	12.4	0
Phosdrin	.50	78.0	0	0		
Thiodan	.50				96.7	82.5
Dieldrin	.25				89.9	87.7
Untreated		93.8				

[a] First application of insecticides was made July 3, but did not include Thiodan and dieldrin. Thiodan and dieldrin were substituted for malathion and Phosdrin, respectively, in the second application of insecticides on July 15.
[b] Based on initial infestation.

Insect infestation records were obtained by counting the immature and adult bugs present on the lower portion of the plants. Samples of plant sections were cut from the stalk and included that part from the ground level up to and including the fourth leaf. Each leaf sheath was removed from the plant section, thus exposing the bugs hidden and feeding behind the sheaths. Insects on the roots and about the ground were not counted.

Results.—Effectiveness of each insecticide against the chinch bug is shown in table 1. Of the sprays included in the first application on July 3, only toxaphene resulted in good control of the chinch bug 9 days following the first treatment. Infestations of chinch bugs 10 days following the second application of toxaphene at 3 pounds per acre were reduced 87%. Three days following one application each of Thiodan at 0.5 pound per acre and dieldrin at 0.25 pound per acre the infestations were reduced 97 and 90% respectively. Ten days following treatments the percentages of reduction were 82 and 88, respectively.

Resistance of the Mite, *Tetranychus cinnabarinus* (Bois.) to Organic Phosphate Acaricides[1]

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In the fall of 1957, cotton growers in the Litchfield Park area of Arizona complained about the failure of organic phosphates to control the mite *Tetranychus cinnabarinus* (Bois.)[2] Observations showed that mite populations continued to build up in spite of repeated applications of demeton and parathion. Organic phosphates had been used extensively in the area for the control of lygus bugs and other insects in practically every treatment applied. In addition, many cotton growers periodically added approximately 2 ounces of demeton per acre as a preventive treatment against spider mites during the growing season. Near this area, the same species of mite on roses was being controlled at that time (September and October 1957) with malathion sprays. A laboratory test was conducted to determine if there was any difference in the control obtained by organic phosphate-acaricides on *T. cinnabarinus* infesting two different host plants.

Laboratory tests were conducted using modified Munger cells as described by Bartlett (1951). Cotton leaves were sprayed with acetone solutions of the acaricides till run-off began. They were allowed to dry before placing in the Munger cell with a piece of moist blotting paper. Ten adult mites were placed in each cell and the test was run for 20 hours. Observations were made at intervals during the test to observe the mortality. In addition, a check was run with each test in which adult mites were placed on untreated leaves. At least three replications were run for each dilution of acaricide for each of the different fields sampled. Mites were considered alive when any movement was observed in the specimen. It was frequently necessary to probe the mites to determine whether they were dead or alive.

Table 1.—The toxicity of various organic phosphate acaricides to *Tetranychus cinnabarinus* collected from "resistant" cotton fields and also from roses. (Laboratory test).

Acaricide	Concentration (p.p.m.)	Per Cent Mortality of Mites Collected from:[a] Resistant Cotton Fields No. 1	No. 2	No. 3	Non-resistant (Roses)
Demeton	1,000	22	—	—	100
	10,000	50	66	71	75
	15,000	91	70	42	100
Parathion	1,000	7	—	—	100
	10,000	12	40	33	67
	15,000	60	37	25	71
Chipman R-6199[b]	1,000	8	—	—	100
	10,000	94	86	91	100
	15,000	99	96	100	100
Untreated		4	3	0	3

[a] Counts made at end of 20-hour exposure.
[b] *O,O*-diethyl *S*-(β-diethylamino) ethyl phosphorothiolate hydrogen oxalate.

The data given in table 1 show that the mites collected from the so-called "resistant" field were much more resistant to demeton and parathion than the same species of mites collected from roses. The same trend is also observed in the Chipman R-6199 test. These data confirm the previous field observations which indicated that the mites in the Litchfield Park area were becoming resistant to organic phosphate acaricides. Mistric (1957) indicated that *T. cinnabarinus* and *Tetranychus telarius* (L.) were the two spider mites most difficult to control on cotton in North Carolina, even with parathion. In the spring of 1958, mites on the roses were apparently becoming resistant to malathion and other phosphates to such a degree that Aramite® 2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite) was used for control.

After the cotton was harvested in one of the mite-resistant fields, beets were planted. In May 1958 this beet field was found to be infested with *T. cinnabarinus*. Spray treatments, indicated in table 2, were applied and replicated three times to plots 0.05 acre in size. The sprays were applied with a tractor-drawn sprayer at the rate of 35 gallons per acre. At periodic intervals afterwards, 10 beet leaves were pulled at random from each plot and the surviving mites in a square-inch-area were counted. The data in table 2 show that demeton did not give commercial control. Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) at 1 pound per acre was more effective than demeton but was not as effective as Aramite. Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol) at the rate of 0.6 pound per acre was almost as effective as Aramite at 1 pound per acre. The best control was obtained with Aramite. Until 1957, demeton was considered superior to Aramite as a control of *T. cinna-*

[1] Arizona Agricultural Experiment Station Technical Paper No. 515, accepted for publication February 16, 1959.
[2] Determinations by U. S. Dept. of Agriculture, Section of Insect Identification and Parasite Introduction.

Table 2.—Control of *Tetranychus cinnabarinus* with various acaricides applied as sprays.[a]

Acaricide	Pounds Toxicant per Acre	Number Mites per Square Inch After: 1 Day	6 Days	14 Days
Demeton	0.375	6.4	9.3	18.4
Trithion	1.0	2.2	4.6	12.1
Kelthane	0.6	11.2	7.6	10.4
Aramite	1.0	11.2	2.9	11.2
Untreated		22.3	18.8	30.9

[a] Applied by ground equipment at 35 gallons per acre.

barinus, indicating again that this mite has developed a resistance demeton.

This laboratory test and the field experiment indicate that *T. cinnabarinus* has developed a resistance to organic phosphate acaricides. This resistance was reported also in California by Andres & Reynolds (1958).

References Cited

Andres, L. A., and H. T. Reynolds. 1958. Laboratory determination of organo-phosphorus insecticides resistance in three species of *Tetranychus* on cotton. Jour. Econ. Ent. **51**(3): 285–7.

Bartlett, Blair R. 1951. The action of certain "Inert" dust materials on parasitic Hymenoptera. Jour. Econ. Ent. **44**(6): 891–5.

Mistric, W. J., Jr. 1957. Chemical control of *Tetranychus telarius* (L.) and *T. cinnabarinus* (Bois.) on cotton. Jour. Econ. Ent. **50**(6): 803–5.

Tobacco Foliage Loss to Hornworms and Budworms in North Carolina, 1957 and 1958[1]

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Data supporting estimates of losses of tobacco to hornworms (*Protoparce sexta* (Johan.) and *P. quinquemaculata* (Haw.)) and budworms (*Heliothis virescens* (F.) and *H. zea* (Boddie)) are meager and based on per cent of leaves injured rather than the actual amount of leaf loss. Hyslop (1938) estimated average annual losses of 5% and 3%, respectively, caused by hornworms and budworms in North Carolina and surrounding states. Morrill (1942) summarized the work of Morrill & Lacroix (1937, 1938, 1939, and 1940). They determined the ratio of injured to uninjured leaves immediately prior to harvest. On sun-grown tobacco (mainly cigar binder), 0.05% to 4.5% of the leaves were injured annually from 1936 to 1940 by hornworms in the Connecticut River Valley. Morrill & Lacroix (1941), the next year in the Connecticut River Valley, found 2.66% of Havana seed tobacco and broad-leaf tobacco injured but only 0.59% with injury of commercial importance. Injury by budworms in the Connecticut River Valley was included with miscellaneous insect injuries of less significance than the hornworm injuries. Shands *et al.* (1938) surveyed North Carolina and adjacent states in August and September, 1935, and found "weighted averages" of 36.9% of the upper leaves injured by hornworms and 9.7% injured by budworms. The manner or size of the "weight" placed on the averages was not indicated. The potential loss of flue-cured tobacco caused by hornworms and budworms averaged more than $28 per acre in 1957 and more than $36 per acre in 1958 (Guthrie *et al.* 1959).

Methods.—Farmers' tobacco fields were surveyed after injury by each of the first and second broods of hornworms in 1958 and only after the second brood in 1957. Feeding by hornworms and budworms was differentiated. Injury by grasshoppers and other large insects was very minor but, if present, was recorded with that of hornworms.

Table 1.—Stratified sampling technique to compensate for tobacco acreage variations of counties.

County Acreage	No. Fields to Be Examined	No. Counties per Class
	Flue-Cured	
Over 30,000	6	3
20,000 to 29,000	5	9
10,000 to 19,000	4	16
1,000 to 9,999	3	29
Under 1,000	None	12
	Burley	
Over 3,000	6	1
2,000 to 2,999	5	1
1,000 to 1,999	4	4
Under 1,000	None	16

Loss estimates were made using quarter-leaf increments as outlined by Lawson & Rabb (Unpublished). The average total number of leaves per plant of flue-cured tobacco was 18. The lower 9 leaves were examined for injury of the first brood of hornworms; the upper 9 for injury of the second brood. Losses to budworms recorded at the same time, represent early-season and late-season injury, as budworms do not have distinct broods.

Stratified sampling, as indicated in table 1, was utilized in 1958 to compensate for variations in tobacco acreages per county. Each survey was begun in the counties of highest tobacco acreages and progressed to counties of lesser acreages. Survey routes usually were main highways. Fields examined were immediately adjacent to the highway and the first fields encountered after passing a predetermined point upon entering the county. In most cases, from one-half to the entire width of the county would be crossed before a sufficient number of fields could be obtained. In counties with an abundance of fields adjacent to the highway, approximately half the samples were taken upon entering the county and half after passing a predetermined point near the center of the county. In no event was a field rejected after arrival at the site, except in case of extreme physical inaccessibility.

Eighty-five plants (1/80 acre of flue-cured, 1/106 acre of burley) were examined in each field. These were examined in 17 groups of 5 adjacent plants. The groups were distributed throughout the field. To avoid bias, the first plant in each of these groups was selected at the termination of a predetermined number of paces, usually 20.

Results and Conclusions.—The data are summarized in table 2. Total losses caused by both hornworms and budworms in 1958 averaged $5.49 per acre of flue-cured tobacco and $3.69 per acre of burley tobacco. Average losses to hornworms totaled $3.35 per acre on flue-cured and $0.67 per acre on burley tobacco during 1958. Losses caused by the second brood of hornworms were nearly 12 times larger than losses caused by the first brood in 1958. The second brood of hornworms caused four times more injury in 1958 than in 1957. Average loss caused by budworms during the season of 1958 totaled $2.14 per acre on flue-cured tobacco and $3.02 on burley tobacco. Early-season and late-season losses caused by budworms were nearly equal on flue-cured tobacco in 1958. Late-season losses caused by budworms in 1958 were similar to late-season losses in 1957. In 1958, losses of flue-cured tobacco caused by hornworms exceeded the losses caused by budworms. The reverse was true for losses of burley tobacco caused by the two kinds of pests.

In an independent experiment comparing insecticide pro-

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Table 2.—Summary of average tobacco leaf loss to hornworms and budworms in North Carolina, 1957 and 1958.

Portion of Plant Examined	Brood of Hornworms Injuring Plants	No. of Fields Examined	Loss Caused by			
			Hornworms		Budworms	
			Leaves/Sample[a]	Dollars/Acre[b]	Leaves/Sample[a]	Dollars/Acre[b]
			1957 Flue-cured			
Upper 9 leaves	Second	50	1.51	0.75	1.60	0.80
			1958 Flue-cured			
Lower 9 leaves	First	121	0.54	0.27	2.11	1.05
Upper 9 leaves	Second	179	6.20	3.08	2.21	1.09
			1958 Burley			
All leaves	First and Second	19	1.13	0.67	5.08	3.02

[a] Sample was 85 plants per field (1/80 acre of flue-cured, 1/106 acre of burley).
[b] One leaf of flue-cured tobacco was valued at $0.0062; burley at $0.0056 per leaf.

grams against tobacco insects on flue-cured tobacco, Baron (1958) reported results that were in close agreement with those reported herein.

With the aid of insecticides, farmers growing flue-cured tobacco in North Carolina in 1958 reduced the potential loss averaging more than $36 per acre to an average of $5.49 per acre. Using the best insecticide available on a biweekly preventive schedule, Guthrie *et al.* (In press) was only able to reduce this loss to an average of $3.51 per acre.

References Cited

Baron, R. L. 1958. *In* Rabb, R. L. and F. E. Guthrie. 1958. Unpublished annual report on tobacco insect research. Department of Entomology, North Carolina State College, Raleigh, N. C.

Guthrie, F. E., R. L. Rabb, T. G. Bowery, F. R. Lawson, R. L. Baron. 1959. Control of hornworms and budworms on tobacco with reduced insecticide dosages. Tobacco Sci. **3**: 65–68.

Hyslop, J. A. 1938. Loss occasioned by insects, mites, and ticks in the United States. U. S. Dept. Agric., Bur. Ent. & Pl. Quar. **E-444.**

Lawson, F. R., and R. L. Rabb. Factors controlling hornworm damage to tobacco and methods of predicting outbreaks. (Unpublished).

Morrill, A. W., Jr. 1942. Insect damage to tobacco in the Connecticut River Valley. Jour. Econ. Ent. **35**(1): 60–62.

Morrill, A. W., Jr., and D. S. Lacroix. 1937. Report of the insect investigations for the 1937 season. Connecticut Agric. Expt. Sta., Tobacco Substa. Rept. for 1937, pp. 444–9.

Morrill, A. W., Jr., and D. S. Lacroix. 1938. Report on the insect investigations for the 1938 season. Connecticut Agric. Expt. Sta., Tobacco Substa. Rept. for 1938, pp. 42–49.

Morrill, A. W., Jr., and D. S. Lacroix. 1939. Report on insect investigations for the 1939 season. Connecticut Agric. Expt. Sta., Tobacco Substa. Rept. for 1939, pp. 186–93.

Morrill, A. W., Jr., and D. S. Lacroix. 1940. Insect investigations during 1940. Connecticut Agric. Expt. Sta., Tobacco Substa. Rept. for 1940, pp. 278–85.

Morrill, A. W., Jr., and D. S. Lacroix. 1941. Connecticut Agric. Expt. Sta., Tobacco Substa. Rept. for 1941, pp. 260–6.

Shands, W. A., Norman Allen and J. U. Gilmore. 1938. A survey of insect injury to tobacco grown for flue-curing. Jour. Econ. Ent. **31**(1): 116–7.

Rotenone and Methoxychlor Dust in Backrubbers for Horn Fly Control in Dairy Herds[1]

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Previous research by Hargett & Turner (1958) indicated that dust formulations of insecticides may be used successfully in backrubbers for the control of horn flies, *Siphona irritans* (L.), on beef and dairy cattle. The materials tested with the backrubbers (25% methoxychlor dust, 10% methoxychlor dust, and 1% Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) dust) are not specifically recommended for the control of horn flies on dairy cattle. Since the dust-charged backrubber was found to be a convenient and economical method of controlling horn flies, it was thought desirable to test formulations that might fall within the dosage range of current recommendations (Entomology Research Division 1958) or other materials which were not likely to result in insecticide residues in the milk.

Materials and Methods.—Two backrubbers were charged with 5% rotenone dust, three with 5% methoxychlor dust, and one each with 4% malathion dust and 25% Delphene® (N,N-diethyl-*m*-toluamide) dust. A single backrubber was employed in each of the herds used in these trials. They were constructed in the manner described by Hargett & Turner (1958) by draping a chain across a cattle walk and wrapping it with three burlap bags. Each bag contained 1 pound of dust formulation. Backrubbers were mounted under shelter in the milking parlor exit so as to force use by each cow.

Daily dosage rates for four of the herds were determined following the last treatment of the season by weighing the amount of insecticide remaining in each backrubber at the end of the experiment. Herd sizes varied from 12 to 60 lactating cows.

An attempt was made at weekly intervals during the experiment to count all of the horn flies on 10 cows in each herd. Untreated herds averaged 100 or more flies per cow throughout the season. Satisfactory control was considered to have been obtained when averages of 10 or less flies per cow were observed. After the horn fly population in each herd built up to an average of 25 or more flies per cow, the backrubbers were rewrapped in new burlap bags and recharged with insecticide.

Results and Discussion.—The effectiveness of the backrubbers, in terms of the number of weeks of satisfactory horn fly control, is shown in table 1. Rotenone dust produced the longest period of control with an average of 6.7 weeks and a range of 6 to

[1] Oregon Experiment Station Technical Paper No. 1207. Accepted for publication February 25, 1959.

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Table 1.—The number of weeks of horn fly control and estimated daily doses of insecticide resulting from use of dust-charged backrubbers.

Material	Herd Number	Date Each Treatment Initiated	Weeks of Control[a]	Estimated Daily Dosage per Cow (oz. of 5% dust)
Methoxychlor 5% Dust	1	8/13	3	—
	1	9/10	5	0.02
	2	9/10	5	.03
	3	7/11	4	—
Rotenone 5% Dust	4	7/10	7	—
	4	9/2	6	.01
	3	8/13	7	.03
Malathion 4% Dust	5	7/16	1	—
Delphene 25% Dust	1	7/11	1	—

[a] An average of 10 or less flies per animal in a 10-cow sample was considered satisfactory control.

7 weeks. Methoxychlor dust gave an average of 4.2 weeks of control with a range of 3 to 5 weeks. Malathion and Delphene each gave 1 week of satisfactory fly control. Weekly averages as low as 0 to 5 flies per cow were observed during the course of each treatment lasting longer than a single week.

Estimates of the daily dosages of rotenone and methoxychlor appear in table 1. The rates of discharge for the four devices varied from 0.01 to 0.03 ounces per day, the average being 0.222.

The current recommendation for the use of rotenone for grub- and louse-control is 3 ounces of 5% dust per animal, applied from one to three times every 30 days (Entomology Research Division 1959). Jacobson and coworkers (1959), using ultraviolet spectrophotometric and paper chromatographic techniques with a sensitivity of 0.04 and 0.006 p.p.m., found no rotenone residue in milk of a cow treated with five times the normal dosage of rotenone for grub control. In view of this fact, there is not likely to be any residue problem when rotenone is applied by the dairy backrubber at the approximate rate of 0.90 ounces of 5% dust per cow every 30 days.

Methoxychlor 50% dust applied at the rate of 1 tablespoon per cow every 3 weeks is recommended for the control of horn flies on dairy cattle (Entomology Research Division 1959), an amount approximating 0.15 ounces of actual toxicant per animal. Five per cent methoxychlor dust, when applied by the dust-charged backrubber, resulted in a dosage of as much as 0.03 ounces of actual methoxychlor per animal over a period of 21 days. Though this dosage is considerably less than the recommended application rate, additional research should be conducted to determine the extent of the residue hazard in milk associated with daily exposure of cows to extremely low dosages of methoxychlor.

References Cited

Entomology Research Division. 1959. Insecticide recommendations for the control of insects attacking crops and livestock. 1959 season. U. S. Dept. Agric. Handb. 121, 129 pp. (p. 118).

Hargett, L. T., and E. C. Turner, Jr. 1958. Horn fly control by the use of insecticidal dusts in self-applicating devices. Jour. Econ. Ent. 51(6): 795–8.

Jacobson, M., J. Hornstein, and R. T. Murphy. 1959. The analysis of milk for rotenoids. Jour. Assoc. Offic. Agric. Chemists 42: 174–6.

Chemical Control of *Tetranychus marianae* McG. on Tomatoes in the Lower Rio Grande Valley[1]

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Tetranychus marianae McG. has become a very important pest of tomatoes in the Lower Rio Grande Valley of Texas. This mite was first reported there in May 1956 by Wene (1956). Since that time the mite has appeared each year in destructive numbers. Commonly recommended insecticides such as malathion and parathion have failed to give adequate control (Wene 1957, 1958). Demeton at 0.25 pound per acre gave good control (Wene 1957), but it cannot be recommended under the provisions of Public Law 518. Adequate control of *T. marianae* on tomatoes was obtained with full coverage sprays of Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), Chlorobenzilate® (ethyl 4,4!-dichlorobenzilate), and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) (Wene 1957, 1958).

Approximately only 20% of the tomato growers in the Lower Rio Grande Valley are equipped for high-volume spraying. Also, airplane applications are necessary during irrigation and after tomato plants begin to overlap in the row middles. As a result, growers have had to use several applications of available dusts against these mites, and the results have not been adequate. Consequently, a highly effective acaracidal dust is needed in the Lower Rio Grande Valley to combat the mite effectively.

Investigations during 1958 involved evaluation of newer acaracides and further evaluation of materials that had given some degree of control the previous year (Wene 1958). Results of these investigations are herein reported.

Materials and Methods.—Dust formulations of malathion, Diazinon, ethion, Kelthane® (1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol), Trithion, and Chlorobenzilate and emulsifiable concentrates of Trithion, ethion, and demeton were used in this investigation. Dusts were applied with a single-nozzle rotary hand duster at the rate of 30 pounds per acre. The emulsifiable concentrates, diluted with water, were applied with a tractor-powered sprayer, having three hollow cone nozzles per row, at the rate of 15 gallons per acre. Applications were made in the early morning or in the evening when the air was calm.

In each test, selected materials were included in a randomized block design with three replications. Plot sizes in the tests were approximately 1/25 acre in the dust tests and $\frac{1}{3}$ acre in the spray test. The first experiment was conducted when tomato plants were 15 to 18 inches tall. Subsequent tests were conducted when tomato plants were mature.

Pretreatment infestation counts and subsequent counts were made by selecting 20 leaflets per plot (10 from each of the two middle rows), brushing the mites from the leaflets with a mite-brushing machine which was developed by Henderson & McBurnie (1943) and trapping them in a 12% aqueous solution of a nonionic alkonolamine condensate (Emcol 5100). A portion of each plate was counted under a binocular microscope with a geometric counting disk (Morgan *et al.* 1955). Percentages of control were calculated by Abbott's formula based on the per cent alive.

The only appreciable amount of precipitation (3.5 inches) occurred on June 27 (third day of test 3). Smaller amounts of rain (light showers of less than 0.1 inch) fell on June 23 (fifth day of test 2) and July 7 (sixth day of test 4 and fifth day of test 5). The daily maximum temperature ranged from 85° to 100° F.

Results.—The results of these tests are presented in table 1. In the first test, demeton, ethion, and Trithion applied as low-volume sprays did not reduce mite populations below damaging

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Table 1.—Control of *Tetranychus marianae* on tomatoes, 1958.

Treatment	Dosage (Lb./A.)	Initial Infestation	Mites per Leaf (A) and Per Cent Control (B) on Days Shown After Treatment					
			Experiment 1—Spray (April 9)					
			5 days		12 days		20 days	
			A	B	A	B	A	B
Ethion	0.5	22.8	23.7	28.7	21.3	71.4	43.8	45.2
	1.0	39.7	37.2	37.4	18.6	81.3	23.6	78.8
Trithion	.5	33.0	28.1	41.9	13.7	86.3	36.4	64.9
	1.0	31.4	27.9	51.8	11.4	89.4	27.3	74.8
Demeton	.25	40.9	33.8	41.5	15.0	90.5	17.4	86.8
Check	—	14.3	25.3	—	47.2	—	65.6	—
			Experiment 2—Dust (June 17)					
			1 day		5 days		7 days	
			A	B	A	B	A	B
Trithion 2	0.6	137.5	54.3	76.2	18.4	95.8	8.8	97.3
Ethion 4	1.2	172.5	48.3	82.3	7.2	97.9	3.2	97.4
Chlorobenzilate 3	.9	112.8	54.9	70.2	34.3	89.1	48.8	64.7
Diazinon 2	.6	106.7	29.2	81.8	68.4	71.3	87.9	51.8
Check	—	91.9	153.1	—	274.0	—	143.1	—
			Experiment 3—Dust (June 24)					
			1 day		6 days			
			A	B	A	B		
Diazinon 1	0.3	152.9	52.5	81.5	40.3	75.2		
Diazinon 2	.6	165.2	36.9	82.2	11.6	95.0		
Kelthane 2.2	.7	177.1	117.1	63.6	12.8	92.3		
Chlorobenzilate 3	.9	159.1	86.3	66.8	28.0	85.3		
Check	—	93.5	163.7	—	96.3	—		
			Experiment 4—Dust (July 1)					
			1 day		3 days		7 days	
			A	B	A	B	A	B
Kelthane 2.2	0.7	70.1	34.0	32.7	29.9	73.9	8.3	74.0
Ethion 4	1.2	87.5	22.1	64.7	4.4	96.9	0.3	99.4
Trithion 2	.6	62.0	32.3	30.1	5.7	93.2	1.5	93.1
Check		64.8	45.5	—	130.7	—	55.7	—
			Experiment 5—Dust (July 2)					
			1 day		5 days		7 days	
			A	B	A	B	A	B
Malathion 5	1.5	19.6	2.7	91.0	23.9	64.9	31.9	46.7
Kelthane 2.2	.7	50.3	14.5	78.3	29.7	77.0	2.1	97.2
Diazinon 2	.6	32.4	1.6	93.9	29.5	44.7	15.7	50.7
Ethion 4	1.2	33.9	2.3	93.6	2.2	97.9	0.3	99.6
Check		25.3	33.5	—	98.2	—	74.5	—

evels, even though each treatment controlled some mites for as ong as 20 days. These results indicated that low volume sprays did not give sufficient coverage for economic control.

Malathion, Diazinon, and Chlorobenzilate were effective in reducing mite populations, but they had poor residual effect. Ethion, Trithion, and Kelthane applied as dusts continued to reduce mite populations for 7 days, indicating residual effect. Ethion and Trithion were superior in this respect.

References Cited

Henderson, C. F., and H. V. McBurnie. 1943. Sampling techniques for determining populations of the citrus red mite and its predators. U. S. Dept. Agric. Circ. 671.

Morgan, C. V., D. A. Chant, N. H. Anderson, and G. L. Ayre. 1955. Method for estimating orchard mite populations, especially with the mite brushing machine. The Canadian Ent. 87(5): 189–99.

Wene, George P. 1956. *Tetranychus marianae* McG. a new pest of tomatoes. Jour. Econ. Ent. 49(6): 712.

Wene, George P. 1957. Commercial control of the tropical mite on tomatoes and the two-spotted spider mite on strawberries. Rio Grande Valley Hort. Soc. Jour. 11: 108–9.

Wene, George P. 1958. Notes on the control of the tropical mite attacking tomatoes. Rio Grande Valley Hort. Soc. Jour. 12: 99–101.

Number of Fall Generations of *Oencyrtus kuwanae* (How.) in Gypsy Moth Eggs

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Oencyrtus kuwanae (How.) has been considered an effective egg parasite of the gypsy moth, *Porthetria dispar* (L.), because of the multiple generations that it can produce in the late summer and early fall. Crossman (1925) stated that probably four genera-

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Table 1.—Parasitism of gypsy moth eggs by *Oencyrtus kuwanae* (How.) at Bethany, Connecticut, in 1958.

DATE	PER CENT OF PARASITIZED EGGS		
	Parasites Emerged	Parasites not Emerged	Total
Sept. 2	2.2	32.1	34.3
Sept. 30	—	—	33.2
Nov. 4	30.4	1.8	32.2
Dec. 2	33.2	0.0	33.2

tions occur from the time the host eggs are deposited until cold weather in the fall halts further activity by the parasite.

In the late summer and fall of 1958, observations were made on the activity of *O. kuwanae* at Bethany, in southern Connecticut. Conclusions reached on data gathered from that location were also borne out by information gained at areas in other parts of the State.

Collections of gypsy moth egg masses were made periodically to determine the percentage of parasitized eggs and to ascertain whether more than one generation per year occurred. This determination was accomplished by counting the number of parasitized eggs from which the adult parasite had already emerged and comparing it with the number of parasitized eggs from which the adult parasite had not issued. To insure an accurate accounting, the eggs which still contained parasites were held at room temperature until all the adults had emerged. An average of 42 egg masses was examined each time.

Results are summarized in table 1. By September 2 very few adult parasites had appeared in the field and the majority of parasitized eggs still contained the parasite larva or pupa. In certain gypsy moth egg masses no parasites whatsoever had issued. As the season progressed, more and more parasites emerged giving the appearance of a rapid increase in the parasite population. The greatest number of adult chalcids was seen during the first week in October. Decreasing numbers of the adults were found on the egg masses in successive weeks until November 18, when the last ones were seen. Shortly after this date, field temperatures dropped below freezing for the first time. A few females were later found in midwinter beneath the ground litter.

Data given in table 1 show that the total percentage of all parasitized eggs remained constant throughout the fall despite the presence of adult parasites. Therefore, the first adults, which started to emerge in late summer, did not produce another generation before cold weather halted all activity.

This failure of further parasitism to take place was not due to a lack of accessible gypsy moth eggs. Although Howard & Fiske (1911) reported that only the uppermost layer of eggs in a gypsy moth egg cluster was attacked, it was found in the present study that a few eggs as deep as the fifth layer were successfully parasitized. However, only the top two layers were heavily attacked. Under optimal conditions in the laboratory, an average of about 55% of the host eggs in intact egg masses was attacked. Occasional individual egg masses in the field reached 50% parasitism. Consequently at least 15% more of the host eggs were available to the parasite but were not attacked. This percentage would be higher in a long, flat egg mass and slightly less in a thick, compact one.

In summarization, the number of adult *O. kuwanae* seen on egg masses of its host is not a reliable indicator of the number of generations. Because the adults can be long lived (Crossman 1925) and can emerge over a considerable period of time, the impression of multiple generations is given; but from the successive sampling of egg masses and total percentage of parasitized eggs, it is clear that there is only one parasite generation from the time the gypsy moth deposits its eggs until warm weather appears the following spring.

REFERENCES CITED

Crossman, S. S. 1925. Two imported parasites of the gypsy moth, *Anastatus bifasciatus* Fonsc. and *Schedius kuvanae* Howard. Jour. Agric. Res. 30(7): 643–75.

Howard, L. O., and W. F. Fiske. 1911. The importation into the United States of the parasites of the gypsy moth and the browntail moth. U. S. Dept. Agric. Bur. Entom. Bull. 91: 1–312.

Reproduction Following Insecticidal Treatment in Two Resistant Strains of House Flies[1]

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An earlier work (Hunter *et al.* 1958) reported the results of a study of the effects upon reproduction of sublethal doses of DDT and Diazinon® (*O,O*-diethyl *O*-)2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) on a DDT-resistant Orlando strain and a susceptible strain of house flies (*Musca domestica* L.). In that work it was found that treatment of the resistant strain with DDT or Diazinon reduced the fecundity, fertility, and offspring survival compared with non-treated flies of the same culture. The overall effect was a 34% and a 21% reduction in the estimated potential offspring for DDT and Diazinon treated females, respectively. In addition, treated females of the resistant strain had a shorter life span than nontreated females.

In the susceptible strain treatment of females with DDT or Diazinon resulted in an increase in some phases of reproduction so that the overall effect was an increase, compared with nontreated flies, of 18% and 1% in the estimated potential offspring production of DDT-treated and Diazinon-treated females, respectively. Treatment had no significant effect upon adult life span in the susceptible strain.

To determine if the above results for the DDT-resistant fly strain were characteristic of that strain only or if similar results might be found in other resistant strains, two additional strains have been studied. These two strains—a DDT-45 strain resistant to DDT, Dilan® (a mixture of 1 part of Prolan and 2 parts of Bulan), dieldrin, and DDVP and a Roberds strain resistant to DDT and dieldrin—were obtained through the courtesy of Dr. Schoof of the U. S. Public Health Service, Savannah, Georgia.

These two strains had been maintained as cultures in our laboratory for three generations prior to the start of the work reported here. The DDT-45 strain had been selected for 218 generations in the U.S.P.H. laboratory at Savannah, Georgia before it was received by us. The Roberds strain was collected from a dairy in 1950 and selected for 196 generations in the same U.S.P.H. laboratory prior to our obtaining the culture used for this work.

The LD_{50} values for these strains for topical application of DDT dissolved in olive oil are as follows: Roberds strain 130 $\mu g/♀$, DDT-45 strain 160 $\mu g/♀$, (Susceptible strain 0.5 $\mu g/♀$). LD_{50} values for topical application of Diazinon dissolved in olive oil are: Roberds strain 0.1 $\mu g/♀$, DDT-45 strain 0.1 $\mu g/♀$, (susceptible strain 0.03 $\mu g/♀$).

The method and techniques for the present study were the same as those used in the previous work (Hunter *et al.* 1958). Briefly, it consisted of treating females of each strain with a

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Table 1.—Records on length of life and reproduction of female house flies treated with DDT or Diazinon and for control females.

Treatment	Female Longevity (Days)	Mean No. of		Per Cent		Estimated Potential Adult Offspring per 100 Females[c]
		Eggs per Clutch	Egg Clutches per Laying Female	Egg Fertility	Progeny Survival to Adult Stage	Females Producing Progeny	
			Roberds Strain				
DDT	15.9[a]	87.8	3.8	66.9[ab]	62.3[a]	73.0[a]	4,322
Diazinon	17.9	88.8	3.7	75.5	69.2	70.0[a]	4,767
Controls	20.6	91.4	4.2	75.8	76.3	87.9	6,302
			DDT-45 Strain				
DDT	18.2[a]	88.4	3.8[a]	67.2[ab]	62.1[ab]	79.7[a]	4,870
Diazinon	19.4[a]	88.0[a]	4.4	76.8[a]	71.4	75.0[a]	4,469
Controls	23.8	93.7	4.8	73.5	72.3	92.4	6,773

[a] Significant difference (P .05 or less) from control flies.
[b] Significant difference (P .05 or less) from Diazinon-treated flies.
[c] Calculated as the mean number of adult progeny per female multiplied by the number of females producing progeny per 100 females.

sublethal dose of DDT or Diazinon dissolved in olive oil and then taking records on individual females for fecundity, egg fertility, offspring survival, and duration of the life span of the treated fly. Similar records were taken on females treated with olive oil only; these flies are referred to as controls. Comparisons of olive oil treated controls and controls not treated with olive oil indicated no effect upon reproduction due to olive oil treatment.

The experiments reported here were carried out over a 6-month period. During this time three separate replicates with each insecticide for both fly strains were completed. In each replicate female flies used for controls and for DDT and Diazinon exposure were taken from the same culture. Following treatment with an insecticide 20 to 30 surviving females were placed, one female per container, in special "ice-cream carton" containers with 1 or 2 males from the same culture. Control flies were handled in the same manner following an application of plain olive oil. The combined results of the separate replicates for each strain are given in table 1. Significant differences between the treated and control flies are indicated in the table.

The results given in table 1 show a consistent response in the relationship of insecticide treatment to mean length of female life. In both strains females treated with DDT or Diazinon had a shorter adult life span than their respective controls, also in each strain the life span of DDT-treated females was less than that of Diazinon-treated females. The mortality was consistently higher in the treated than control flies, in the first week following treatment. After the first week the mortality of treated and control flies was generally about equal.

The treated females in each strain laid fewer eggs per clutch than their respective controls; however, the decrease in fecundity was not particularly large in either strain and was almost identical within each strain for the two insecticides. In both strains the treated females laid eggs over approximately the same time period and at the same frequency as the controls. The slightly higher number of egg clutches per laying female in the controls may have been, at least partially, due to the longer life span of these flies.

The most striking difference in response between the treated flies of the two strains was in egg fertility. The Diazinon-treated females of the DDT-45 strain showed a significantly higher egg fertility than their control females; in contrast to this, Diazinon-treated females of the Roberds strain showed no difference from their controls in egg fertility. DDT-treated females of each strain laid fewer fertile eggs than their controls or than Diazinon-treated females. Larval and pupal survival of both DDT- and Diazinon-treated flies was lower than that of their controls, but was lowest in each strain in DDT-treated females.

The egg laying period was divided into three approximately equal periods to determine whether any unusual variation occurred in the rate of decline in fecundity or fertility. The rate of decline in the mean number of eggs per clutch was approximately the same in the treated and control flies of each strain. However, egg fertility declined slightly faster in the first part of the laying period in treated than in control females. The reverse was true for egg fertility in the last half of the laying period.

The sex ratio for adult flies reared from treated females was not significantly different from a 1:1 male to female ratio except for DDT- treated females of the Roberds strain in which there were significantly fewer female progeny produced.

From records of offspring survival it was possible to calculate the average number of adult progeny produced per female and from this to estimate the progeny 100 females would produce (all 100 females would not produce offspring—see per cent females laying eggs, table 1). The per cent decrease in the estimated offspring production of 100 treated compared with control females is given in table 1. The reduction in the estimated potential adult offspring from treated females was largely due to the fact that in each strain fewer treated females laid eggs.

The reduction in the estimated potential adult progeny given in table 1 is of the magnitude reported previously for the Orlando strain; however, some minor differences between the two studies occur in the various stages of reproduction. This was apparent in a higher percentage of females laying eggs in the Orlando strain, and in a higher per cent egg fertility in the Diazinon-treated compared to control females of the DDT-45 strain.

Additional tests on other susceptible strains would also have been desirable; but, because of the necessary time involved, such tests were not practicable.

In summary, the results of the present experiment show a similar response for the two DDT-resistant strains studied. Except for egg fertility of the DDT-45 Diazinon-treated females, the treated females of each DDT-resistant strain showed some reduction in all phases of reproduction compared with control females. We have no valid explanation for this. Lack of vigor has been suggested, but some more specific factor or factors may be responsible. The general results of the present work are in agreement with those reported for the DDT-resistant Orlando strain in an earlier paper.

Reference Cited

Hunter, P. E., L. K. Cutkomp, and A. M. Kolkaila. 1958. Reproduction in DDT- and Diazinon- treated house flies. Jour. Econ. Ent. **51**: 579–82.

A Comparison of Three Methods of Insecticide Application for Control of the Rice Water Weevil[1]

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The larval stage of the rice water weevil, *Lissorhoptrus oryzophilus* Ksl. is one of the most important insect pests attacking rice in the Southern United States. Whitehead (1954) obtained adequate control of this insect by making spray applications of various insecticides to the soil and plants just prior to applying the first flood water. Bowling (1957) found that various insecticides applied to the seed prior to drilling would control the larval stage of this insect.

In Texas, it is a common practice to apply at least part of the fertilizer just prior to applying the first flood water. The date of application usually coincides with the proper timing for control of rice water weevil by spraying. It was theorized that if an insecticide-fertlizer mixture could be used successfully, the cost of application would be reduced. An experiment was conducted at the Rice-Pasture Experiment Station, Beaumont, Texas in 1958 to compare spray applications with seed treatments and with two fertilizer-insecticide mixtures for controlling this insect.

EXPERIMENTAL PROCEDURES.—A split plot experimental design was used with plots 4′×20′, replicated four times, The treated plots with each method of application received 8 ounces of technical aldrin per acre. A long grain variety, Century Patna 231, was used in this test. Seed treatment plots were drilled with seed treated with a slurry made by adding water to a 50% wettable powder. This was poured over the seed which were in a 1-gallon glass jar. The jar was then closed and shaken by hand until uniform coverage was obtained. The spray treatments were applied on dry soil when the rice was approximately 3 weeks old. All plots in the experiment were fertilized at the rate of 60–40–0 per acre using 33% ammonium sulphate and 45% superphosphate. In the insecticide-fertilizer treatments, the insecticide was mixed with the fertilizer before application. In one treatment, a wettable powder (50%) was used, and in another, a granulated (10%) was added. The fertilizer-insecticide mixtures were applied on the same date spray applications were made. The test plot was flooded immediately after treatments were made and remained flooded until harvest. Larval counts were made 3 weeks from date of first flooding. Counts were based on the number of larvae per foot of drill row. A sampling tool designed to take a sample of plants, soil and roots 1′×4″×4″ was used. This sample was then examined as described by Whitehead (1954). Yields are reported in pounds of rough rice per acre.

RESULTS.—Data reported in table 1 indicate that all methods of application were equally effective in controlling rice water weevil larvae. Although larval infestations were light, highly significant yield increases were obtained from all methods of application.

DISCUSSION.—If further testing of insecticide-fertilizer mixtures prove this method effective, it will provide rice growers with a choice of three methods for controlling the rice water weevil. The cost of insecticide is approximately the same in each method used. The main difference is in the cost and convenience of application. Each method has advantages and disadvantages when used in conjunction with various cultural practices.

Seed treatment is practical and convenient; especially, if the grower already uses a fungicidal seed treatment, as the two treatments can be made in one application. Seed treatment also has the advantage of protecting the seed rice from storage insects and the seedling rice from various soil insects. However, seed treatment does not control the adult rice water weevil. This method of application has not been tested for water seeded rice.

Spray applications are effective and have the advantage of killing other minor insects that might be present in the field at the time of application. However, the cost of applying a spray is considerably more than applying seed treatment or fertilizer-insecticide mixtures. Also, spray applications must be made on dry soil. This, sometimes, is impossible owing to rains and various cultural practices used.

Insecticide-fertilizer mixtures have the advantage of low cost of applications. However, they have the disadvantage of having to be custom-mixed for the individual rice grower in Texas. Also, it is not always feasible or possible to apply the fertilizer at the proper time for water weevil control.

Table 1.—A comparison of three methods of insecticide application for rice water weevil control.

METHOD OF APPLICATION	LARVAE PER FOOT OF DRILL ROW	PER CENT CONTROL	YIELD (LBS./A.)
Seed treatment	2.7	90.6	3,478
Untreated check	29.0		3,254
Spray application	4.0	83.6	3,350
Untreated check	24.2		3,201
Fertilizer+WP[a]	2.7	89.9	3,355
Untreated check	27.0		2,982
Fertilizer+Gr.[b]	5.2	69.2	3,309
Untreated check	17.0		3,042
Average (treated)	3.6	85.0	3,373
Average (untreated)	24.3		3,119
L.S.D. at 5% level	8.1		132
at 1% level	11.7		191

[a] Wettable powder (50%) mixed with fertilizer.
[b] Granulated material (10%) mixed with fertilizer.

REFERENCES CITED

Bowling, C. C. 1957. Seed treatment for control of the rice water weevil. Jour. Econ. Ent. 50(4): 450–2.

Whitehead, F. E. 1954. Tests on insecticidal control of rice water weevil. Jour. Econ. Ent. 47(4): 677–80.

[1] Accepted for publication March 13, 1959.

Egg Relationships of *Drosophila* in Processed and Unprocessed Tomatoes in New Jersey[1]

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The vinegar fly (*Drosophila* spp.) problem, both in tomato fields and around canneries, has been reported by Pepper *et al.* (1953), Michelbacher & Middlekauff (1954), and others. These flies are usually most abundant in New Jersey from about the middle of August until the end of the tomato season, with the result that tomatoes reaching the cannery during this period are often heavily infested with fly eggs and maggots. To date, field control has been unsatisfactory, and efforts in New Jersey to successfully curtail egg-laying on split tomatoes have been only partially effective.

The problem with *Drosophila* is primarily one of fly eggs in the finished tomato product. An attempt was made to determine what relationships existed, if any, between eggs of *Drosophila* occurring in harvested tomatoes and those found in finished tomato products. This detailed study was conducted during the season of 1956.

PROCEDURES.—Tomatoes which had natural growth cracks and which had callused over in the field were selected as the first

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers—The State University of New Jersey, Department of Entomology. Accepted for publication March 13, 1959.

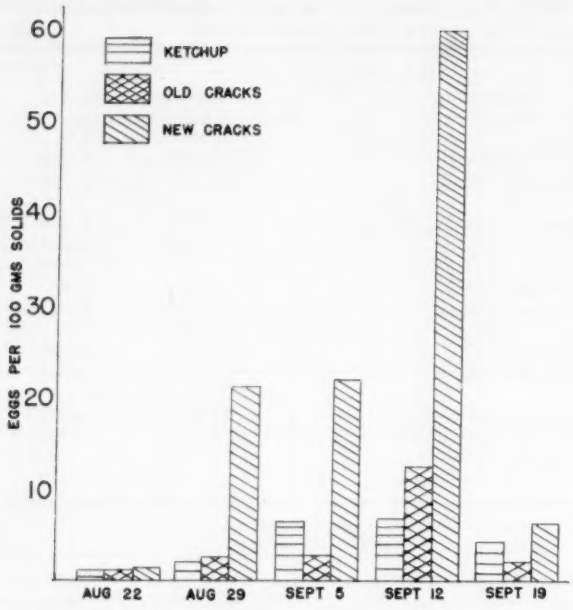

FIG. 1.—*Drosophila* eggs in processed tomato pulp and ketchup subsequent to exposure to infestation in the field.

type to study, since it seems probable that eggs laid in such cracks would contribute eggs to the finished product. These fruits, referred to here as "old cracks" commonly occur in wet growing seasons. The other type selected for study was fruit which had been mechanically cracked or split in the process of picking, loading, and unloading. This type will be referred to as "new cracks" in this report. The finished product used for comparison was ketchup.

The data on eggs was obtained in the following manner:

1. A basket of naturally cracked fruits was picked from a selected commercial field at the same time the regular harvests were being made at weekly intervals for a period of 5 weeks.

2. During the same time period a basket of mechanically cracked tomatoes was taken from a truck-load that had been transported to the canhouse from the above field.

3. A sample of ketchup from the regular factory run was obtained so as to coincide with the time the truck was unloaded. This sample gave only an approximate comparison for a particular load, but it was felt it would have sufficient validity for the given time range.

4. The tomatoes from samples 1 and 2 were processed into juice in the Department of Food Science, New Jersey Agricultural Experiment Station, using the following technique: The tomatoes were soaked in water at 65° F. for 3 minutes and washed under running water 1 minute. All observable mold and rotten and green spots were trimmed; the fruits were quartered into 6-quart pots and heated almost to boiling (205° to 208° F.). The heated pulp was put through the pulper using a 0.027-inch screen, cooked, packaged in fiber freezer containers, and frozen at −10°F.

5. The weight of ketchup taken for sampling was in all instances 100 gms. and the sample of tomato juice was adjusted to a weight of 218 gms. This is the approximate ratio of solids of ketchup to juice.

6. Eggs in the samples were determined by the Offical Methods of Analysis of the Association of Official Agricultural Chemists (1955).

RESULTS.—This study showed that there was a high percentage of eggs eliminated during processing.

It is obvious from the data shown in figure 1 that new cracks contained by far the largest number of eggs. This suggests that the number of eggs in the finished product, which closely parallels the increase in new cracks, could be decreased if more attention were given in the field to careful picking and handling.

Percentagewise the old cracks contributed more larvae than new cracks, since eggs deposited in these slits may become sealed over and still develop into the larval stages. This would seem to follow since it is possible for eggs in cracks to become sealed over, but still develop into the larval stages. Old cracked tomatoes contribute a smaller number of eggs to the finished product, since they are trimmed more closely than newly cracked fruits.

REFERENCES CITED

Pepper, Bailey B., J. P. Reed and O. Starnes. 1953. Drosophila as a pest of canning tomatoes. Ext. Bull. 266, College of Agriculture, Rutgers University.

Michelbacher, A. E., and W. W. Middlekauff, 1954. Vinegar fly investigations in northern California. Jour. Econ. Ent. **47:** 917–22.

Official Methods of Analysis of the Association of Official Agricultural Chemists. 8th edition, 1955, page 783–35. 63.

Preliminary Study of European Pine Shoot Moth Parasitism in Lower Michigan[1]

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To obtain the background necessary for deciding whether foreign parasites of the European pine shoot moth, *Rhyacionia buoliana* (Schiff.), should be introduced into Michigan, a survey was made in 1958 to find out how much the insect is now being affected by parasites. Two shoot moth problem areas in Michigan were investigated: Ottawa and Wexford Counties. Observations were made at various times to include the egg, larval, and pupal stages of the host. Larval or pupal populations of the shoot moth in some of the pine plantations sampled averaged from less than 1 up to 38 insects per tree, based on May counts. The trees in all the plantations were from 3 to 9 feet tall. Collections were taken at random from all parts of the trees. Each of the study plantations was sampled only once.

Parasitism of eggs, all apparently caused by *Trichogramma minutum* (Riley),[3] averaged 4% (table 1). This is a cosmopolitan parasite which attacks the eggs of many other insects. Its abundance as a shoot moth parasite probably depends on the density of other suitable hosts in the area.

The first larval parasite species to complete development transformed to the pupal stage in August and September. This was the gregarious external *Hyssopus thymus* (Girault). Its frequency of occurrence in the summer averaged 6% of the injured tips (table 2). *H. thymus* developed another generation on the shoot moth the following spring.

In the spring, several parasite species were found, but the degree of parasitism was not greater than before, the average being 4% (table 3). In descending order of abundance, the spring parasites were *Hyssopus thymus, Itoplectis conquisitor* (Say), *Calliephialtes comstockii* (Cresson), and *Eurytoma pini* Bugbee (formerly *tylodermatis* (Ashmead)—see Bugbee 1958). *Itoplectis conquisitor* is a solitary internal pupal parasite while *Calliephialtes comstockii* and *Eurytoma pini* are solitary external larval

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[2] Stationed at the East Lansing, Michigan, field unit, which is maintained in cooperation with Michigan State University.

[3] Adult parasite specimens were identified by specialists as follows: Ichneumonidae by L. M. Walkley of the Insect Identification and Parasite Introduction Laboratories, U. S. Department of Agriculture; Eulophidae and Trichogrammatidae by B. D. Burks, of the same Laboratories; and Eurytomidae by R. E. Bugbee of Allegheny College, Meadville, Pennsylvania.

Table 1.—Parasitism of eggs, 1958.

County[a]	Species of Pine	Number of *R. buoliana* Eggs Examined	Percentage Parasitized
Ottawa	Red, *Pinus resinosa* Ait.	347	1
Ottawa	Scotch, *P. silvestris* L.	180	2
Wexford	Red	115	16
Total or average		642	4

[a] One plantation in each county. *Trichogramma minutum* apparently was the only parasite involved.

Table 2.—Parasitism of young larvae in the summer, 1957.[a]

County	Number of Plantations Sampled	Species of Pine	Number of Injured Tips Examined[b]	Percentage Parasitized
Ottawa	1	Red	50	4
Wexford	5	Red	342	6
Total or average	6		392	6

[a] Parasite counts were made during the winter of 1958; *H. thymus* pupae overwinter in the shoot moth larval tunnel and can be easily detected during this season. Apparently it was the only parasite involved.

[b] Estimated that 1 living larva per tip occurred at time of parasitization in the summer.

Table 3.—Parasitism of older larvae and pupae by all species of parasites in the spring, 1958.

County	Number of Plantations Sampled	Species of Pine	Number of *R. buoliana* Examined	Percentage Parasitized
Ottawa	4	Red	1162	3
Ottawa	4	Scotch	463	6
Ottawa	2	Austrian, *Pinus nigra* Arn.	179	3
Ottawa	2	Jack, *P. banksiana* Lamb.	60	8
Wexford	4	Red	306	5
Total or average	16		2170	4

parasites. All except *Hyssopus thymus* are univoltine on the shoot moth, and all are native species.

The degree of parasitism and the parasite species found thus far in Lower Michigan are very similar to those reported for the shoot moth from other areas of the eastern United States and Canada (Friend & West 1933, Coppel & Arthur 1953, Miller & Neiswander 1955).

In Europe, pine shoot moth populations tend to be lower[4] and parasitism tends to be higher (Neugebauer 1952) than in Michigan and other areas in North America. Also, many more species of parasites attack the shoot moth in Europe than in North America. These facts suggest that introduction of European parasites of the shoot moth into Michigan and wherever else they do not already occur would be worth while.

References Cited

Bugbee, R. E. 1958. A new species of *Eurytoma* Illiger, parasitic on the Nantucket pine moth, *Rhyacionia frustrana* (Comstock) and the European pine shoot moth, *R. buoliana* (Schiffermuller). Jour. Kansas Ent. Soc. **31:** 197–200.

Coppel, H. C., and A. P. Arthur. 1953. Notes on introduced parasites of the European pine shoot moth, *Rhyacionia buoliana* (Schiff.) (Lepidoptera: Tortricidae), in Ontario. Ent. Soc. Ontario, Ann. Rept. **84:** 55–58.

Friend, R. B., and A. S. West. 1933. The European pine shoot moth (*Rhyacionia buoliana* Schiff.) with special reference to its occurrence in the Eli Whitney Forest. Yale Univ. Sch. Forest. Bull. **37:** 1–65.

Miller, W. E., and R. B. Neiswander. 1955. The biology and control of the European pine shoot moth. Ohio Agric. Expt. Sta. Res. Bull. **760:** 1–31.

Neugebauer, W. 1952. Die Bekaempfung des Kieferntriebwicklers. Forstarchiv **23:** 159–65.

[4] Data available at the Lake States Forest Experiment Station, East Lansing, Mich.; manuscript in preparation by W. E. Miller.

Further Comparative Toxicity Tests against the Western Cherry Fruit Fly[1]

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This paper continues the comparative toxicity tests conducted with selected pesticides available in 1954 and 1955 using the western cherry fruit fly, *Rhagoletis cingulata indifferens* Curran, as the test insect (Frick, 1957). Because large numbers of heavily infested cherries were available during the summer of 1957, it was decided to test several of the newer materials during the winter of 1958, even though the western cherry fruit fly has decreased in importance in eastern Washington as a result of properly timed yearly spray schedules.

MATERIALS AND METHODS.—The techniques and the Hoskins-Caldwell spray chamber described by Frick (1957) were employed without change. Parathion, Korlan®, Dibrom®, and Guthion® were tested with an average of 788 flies each after the dosage-mortality ranges had been determined in preliminary tests. Each of the four materials was replicated four or five times at each concentration. Trithion®, Thiodan®, and ethion were given preliminary tests to determine the toxicity range of each in relation to parathion. A total of 5,475 flies was used in these trials.

All insecticides were emulsifiable concentrates and are listed in order to show the percentage of active ingredient and the company supplying each.

Dibrom (RE 4355) (*O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate), 63%, California Spray-Chemical Corporation

ethion(Nialate), 47.1%, Niagara Chemical Division.

Guthion (*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3,-benzotriazine-3-methyl) phosphorodithioate), 18.4%, Chemagro Corporation

Korlan (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate), 24,4% Dow Chemical Company

parathion, 25%, Yakima Valley Spray Company

Thiodan (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), 24%, Niagara Chemical Division

Trithion (*S*-*p*-chlorophenylthio) methyl (*O,O*-diethyl phosphorodithioate), 25%, Stauffer Chemical Company.

RESULTS AND DISCUSSION.—The four materials that were adequately tested are compared in table 1. The dosage values were taken from eye-fitted regression lines. The toxicity index of Sun (1950) is employed to give a direct comparison of each insecticide with parathion. Because of the 3-year lapse since the previous tests, parathion was retested. The dosages varied slightly from those of 1955 (Frick 1957) but this may have been caused by the different formulation used. As a contact spray, Korlan was very close to parathion with the regression lines crossing at LD_{75}. Guthion was about one-half as effective. Dibrom, a halo-

[1] Scientific Paper No. 1827, Washington Agricultural Experiment Stations, Pullman. Project No. 791. Accepted for publication March 20, 1959.

Table 1.—Comparative toxicities of several insecticides to the cherry fruit fly. Prosser, Washington, 1958.

Insecticide	LD_{50} ± Actual Insecticide	Toxicity Index	LD_{95} % Actual Insecticide	Toxicity Index
Dibrom	0.0085	220	0.0245	257
Parathion	.0187	100	.063	100
Korlan	.018	104	.068	93
Guthion	.043	43	.13	48

genated phosphate with a very short residual value, was more than twice as toxic. Dibrom would appear to be useful for sprays just prior to or during harvest when a quick knockdown of flies frequently is required and no toxic residues can be tolerated.

Trithion, ethion, and Thiodan were each tested once after preliminary trials to establish dosage limits. Thiodan and ethion were about one-fourth as toxic as parathion, having toxicity indices of 25 and 27. Trithion had a very steep regression line with a toxicity index at LD_{50} of 34 or about one-third that of parathion, while that at LD_{95} was 69 or about two-thirds that of parathion. The toxicity of Thiodan and of ethion was considered too inferior to that of parathion to warrant further testing. Trithion should be considered in future testing because of its superior toxicity at higher dosages.

Field Trial.—Because Korlan compared so favorably with parathion as a contact spray in the laboratory, it was given a field test. The orchard chosen was one at the Experiment Station with a history of heavily infested cherries. Two replications were used. Each replication consisted of a row of mixed sour and sweet varieties. Between each sprayed row was an unsprayed row. Each cherry row was separated from the next cherry row by two or three rows of other stone fruits.

The first fly was found on May 16 and sprays consisting of 1 pint or 0.25 pound active parathion or Korlan per 100 gallons were applied on May 20, June 2, and 11. A high-pressure orchard sprayer was used. The sprays were applied with hand held guns and the trees were drenched to the point of heavy runoff. Cherry fruit fly adults were common in the trees beginning on May 28, and bait trap records showed that the peak of flight activity occurred on June 4. Many flies were seen in the Korlan-sprayed trees beginning 2 or 3 days after each application, though they were very scarce in the parathion-sprayed trees.

Samples were taken on June 21 by picking 200 fruits at heights of 5 to 7 feet above the ground from the trees in each row. Parathion proved to be far superior to Korlan, the two replications each having 2.5% of the fruit infested. Korlan-sprayed fruits were 8.5 and 16% (average 12%) wormy, while fruits from three check rows were 19.5, 29.5, and 50.5 (average 33)% infested. Korlan apparently lacked residual value under these orchard conditions.

References Cited

Frick, K. E. 1957. Comparative toxicity tests as an aid in selecting insecticides for control of the cherry fruit fly. Jour. Econ. Ent. **59**(3): 256–9.

Sun, Y. P. 1950. Toxicity index—an improved method of comparing the relative toxicity of insecticides. Jour. Econ. Ent. **43**(1): 45–53.

The Cotton Rat Irruption in Dallas, Texas in 1958[1]

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The Vector Control Section of the City of Dallas Public Health Department has studied and evaluated the City's endemic typhus potential in house rats present within the City limits of Dallas for several years. The results of the first City wide survey, begun in January, 1956 and continued through March, 1957, showed 2.68% of 76 roof rats infected with murine typhus. No endemic typhus infection was found in 181 Norway rats. Only 10% of all rats trapped were infested with the oriental flea, *Xenopsylla cheopis* (Rothsch.), the endemic typhus vector (Laake 1957).

As a part of the Public Health Department's routine surveillance program, the typhus-in-rat survey in the City of Dallas was resumed during the period extending from November 10, 1957 to March 10, 1958. The object was to determine the prevailing murine typhus potential in the City. For this survey more rats were trapped in the immediate areas of the two locations where typhus infected rats were found during the 1956–57 survey than in other areas of the City, primarily to learn what degree of infection remained in these areas. However, all areas of the City were included in the survey in order to obtain a true picture of the typhus potential of the City as a whole.

Blood from 118 Norway rats and from 10 roof rats trapped during the 1957–58 survey was negative for typhus by the complement fixation test and only 7% of all rats trapped were found to be infested with oriental rat fleas, indicating a decrease in flea abundance and a possible disappearance of typhus in rats in the City during 1957. The reason for the apparent disappearance of typhus in rats in the City is unknown.

An irruption of cotton rats (*Sigmodon hispidus* Spp.) in the fall of 1958 in the outlying areas in the northern section of Dallas precipitated a great demand for information on the disease-bearing potentialities of this species of rat from both the medical profession and especially from residents in this area. *S. hispidus* apparently is the only species of cotton rat found in the Dallas area. Although these rodents have not been incriminated as reservoirs of endemic typhus, there is always the possibility that they might pick up domestic rat fleas and become infected, even though we consider this unlikely. Because of the tremendous numbers of cotton rats that were present in the outward areas of the City and especially in the rural areas bordering the City where no typhus-in-rat surveys have been made during recent years, we deemed it important to determine whether these rats were carriers of endemic typhus.

Our Vector Control Section trapped nearly 300 cotton rats in the City during the period from September 25 to December 5, 1958. The rats were trapped in grass and weed-covered vacant lots adjoining yards in the outlying areas of the City but not in homes because this rat usually does not invade dwellings. Blood obtained from over 100 of the larger individuals trapped was found to be negative for typhus as determined by the State of Texas Department of Health.

From 199 cotton rats of all ages, which were combed for ectoparasites, no oriental rat fleas were found. Among 6,492 specimens of other ectoparasites combed from the rats, four species of mites were present, 3 of fleas, 1 of lice and 1 of ticks. The various species present on the rats and their relative abundance are listed in table 1. We are deeply grateful to Dr. Richard B. Eads and his staff, Section of Entomology, State of Texas Department of Health for all the determinations of the ectoparasites.

Previous investigations by Public Health Organizations have demonstrated that this rodent is not known to be a reservoir of of any importance for disease agents transmissible to man. Dr. J. V. Irons, Director of Laboratories, State of Texas Department of Health, in a letter dated December 15, 1958, stated, "We have always given the cotton rat a clean bill of health as a potential typhus reservoir. Since cotton rats associate so little with human beings, it is doubtful that they present much of a threat to the public health." The negative results of the Dallas typhus-

[1] Accepted for publication March 23, 1959.

Table 1.—Ectoparasites found on 199 cotton rats (Sigmodon hispidus) trapped in Dallas, Texas during the period September 25 to December 5, 1958.

Ectoparasites	Rats Infested (%)	Average No. Parasites Per Infested Rat	Average No. Parasites On All Rats
Fleas			
Ctenocephalides felis (Bouche)	0.5	6.0	0.03
Orchopeas leucopus (Baker)	4.5	3.2	.15
Polygenis gwyni (C. Fox)	0.5	1.0	.01
Lice			
Hoplopleura hirsuta Ferris	41	6.3	2.71
Mites			
Echinolaelaps echidninus Berlese	0.1	1.7	0.03
Haemolaelaps glasgowi (Ewing)	92.4	30.2	27.44
Listrophonidae	1.5	3.3	0.22
Ornithonyssus bacoti Hirst	65.8	11.2	6.36
Ticks			
Dermacentor variabilis Say	1.5	1.3	0.02

in-cotton rat survey confirm those obtained elsewhere, conducted earlier by the State of Texas Department of Health and other Public Health Organizations. We feel, therefore that the control of this rat is not a primary public health responsibility. However, we do feel that all health departments should be in a position to advise property owners as to proper procedures to follow in controlling cotton rats as well as other local species of rats. In Dallas, the City Health Department used the local television and radio facilities, and a circular prepared by the Department, to inform residents in the rat-invaded areas that this rat is not known to be a carrier of endemic typhus and to recommend approved methods for its control.

Reference Cited

Laake, Ernest W. 1957. A city surveys its endemic typhus potential. Texas Public Health Assoc. Jour. 9(4): 156–9.

Japanese Beetle Appraisal Survey in Outlying Areas, 1958[1]

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Agric. Res. Serv., U.S.D.A.

Japanese beetle (*Popillia japonica* Newm.) was first discovered in the United States at Riverton, New Jersey in 1916. Since then the pest has spread gradually into 16 states, as far west as eastern Ohio, as far south as northern South Carolina, and as far north as southern Maine. A State-Federal regulatory and control program, designed principally to retard long-distance spread, was established in 1919. Despite this effort, the beetle has spread by artificial means to isolated spots in nine additional states surrounding the generally infested area. During the past decade several of the isolated occurrences have developed into consequential infestations. This situation led to a resolution by the Central Plant Protection Board in early 1958 asking the United State Department of Agriculture to conduct an appraisal survey in the areas west and south of the federally regulated area to collect data on which corrective measures could be based.

The Department's Plant Pest Control Division undertook this survey in cooperation with the 14 affected states during the summer of 1958. The work involved increased survey activities employing over 30,000 traps as well as visual inspection where applicable. In addition, special field studies were conducted in the more important areas and interviews were held with over 100 State and Federal regulatory, research and extension officials.

The survey showed that Japanese beetle infestations were widely scattered in the outlying states. The insect was found to occur in 118 counties south and west of the federally regulated areas. These isolated infestations involved 13 states,[2] four of which (Virginia, West Virginia, Ohio and North Carolina) were under Federal quarantine and portions of the State regulated. Delimiting surveys revealed an estimated 1,338,000 acres infested in six states: South Carolina, Georgia, Kentucky, Ohio, Illinois and Indiana. Nonregulated infestations in North Carolina, Virginia and West Virginia were considered too extensive and complex to delimit practicably. A relatively small infested acreage was found in the remainder of the states surveyed: Iowa, Michigan, Missouri and Tennessee. It should be noted that all infestations in this latter group have been treated.

Thirty-one new locations, exclusive of single beetle collections which are not considered indicative of established infestations, were reported during the year. These data strongly indicate the need for increased detection efforts in regions where the pest is not known to occur in order that requirements for regulatory and control measures can be determined.

Japanese beetle adult emergence was generally late in 1958 and populations were less than normal in the eastern and lower Great Lakes regions but about normal in the South Atlantic

[1] Accepted for publication April 3, 1959.
[2] No beetles were found in Wisconsin in 1958.

Table 1.—Summary of Japanese Beetle Survey and Control Outside Regulated Areas, 1931–1958.

Location of Infestation	First Year Found	Major Areas 1958	Population Density 1958	Estimated Acres	Acres Treated 1958	Cumulative Acres Soil Treated
Georgia	1937	Dahlonega, Atlanta	Light to heavy	248,960	44	1,168
Illinois	1934	Sheldon, Chicago, E. St. Louis	Light to heavy	103,425	12,728	25,216
Indiana	1934	Kentland, South Bend, Logansport, Indianapolis, Terre Haute, Elkhart	Light to heavy	204,529	99	8,872
Iowa	1957 (Estab.)	Ft. Madison	142 beetles collected	Treated	2,048	2,048
Kentucky	1936	Pikeville, Louisville, Covington	Light to heavy	220,243	2,306	5,969
Michigan	1932	Detroit	164 beetles	Treated	212	5,090
Missouri	1934	St. Louis	92 beetles	Treated	160	1,056
Ohio[a]	1931	Cincinnati, Chillicothe	Light to heavy	230,631	166	9,945
South Carolina	1931	Northwestern Counties	Light to heavy	330,300	0	54
Tennessee	1936	Knoxville, Butler	Light	Treated	2,359	3,227
Wisconsin	1956[b]			0	0	4
Totals				1,338,288	20,122	62,649

[a] Figures are for nonregulated area only. Eastern counties under Federal regulation.
[b] One beetle found in 1956; none in 1958.

States. Although the populations in some of the isolated locations were heavy, most could still be classified as light and scattered. Only 164 beetles were captured in 89 of 9,175 traps operated in Michigan and most of these were in the Detroit area. Ninety-two beetles were collected at St. Louis, Missouri and 142 beetles at Fort Madison, Iowa. These were the only known infested locations in the two states. Populations were light in most areas of Illinois, Kentucky and Tennessee also. The heaviest isolated infestations, those in which plant injury was readily noticeable, were at Cincinnati, Ohio; Pikeville, Kentucky; Dahlonega and Atlanta, Georgia; and in northwestern South Carolina. Several locations adjacent to regulated areas in North Carolina and Virginia also had numerous beetles and heavy foliage injury. No significant amount of adult or larval damage was observed or reported in any location, however.

These observations indicate that while the Japanese beetle is regarded in the outlying areas as a highly important pest from the regulatory standpoint and is often referred to as a potential major crop pest, most complaints received by entomologists and extension personnel in the nonregulated areas are from homeowners who report damage to ornamentals.

Table 1 summarizes the major aspects of the Japanese beetle situation in the states under discussion, except in the nonregulated areas of West Virginia, Virginia and North Carolina.

Malathion Dust for the Control of Two Species of Pigeon Lice[1]

J. L. RODRIGUEZ and L. A. RIEHL, *University of California Citrus Experiment Station, Riverside*

Squab raising in southern California represents a small but important monetary facet in the poultry industry. Also, this fowl is often kept in small backyard flocks as a source of meat for the family table.

In this area, pigeons are commonly infested with the slender pigeon louse, *Lipurus baculus* (Nitzsch), and the small pigeon louse, *Goniocotes bidentatus* (Scop.). When conditions are favorable, infestations of lice can grow rapidly and create a stress to the pigeons from the constant irritation. Accepted treatments, Lee & Haynes (1915), have consisted of dusting by the pinch method or dipping with sodium fluoride. Both techniques are tedious and have high labor costs. In addition, sodium fluoride often causes irritation of the nose and throat. Our observations indicate many growers prefer disregarding louse control to using the sodium fluoride treatment. Therefore, investigation of newer pesticides and treatment techniques seemed desirable.

MATERIALS AND METHODS.—Two ranches (A and B) with small pigeon colonies were selected for trials of malathion dust. The birds were infested with the slender pigeon louse and the small pigeon louse. Control of these species of lice had never been attempted at either ranch. At Ranch A, 100 birds were hand-dusted with a small duster with 1% malathion dust. Each bird was dusted once on the ventral and dorsal surface of the wings, on the back, base of the tail, the breast and in the fluff. The dust was directed against the natural lay of the feathers to force the dust under the feathers and onto the skin.

At Ranch B, 0.5% malathion dust was applied as above to 30 birds. A comparable group of 30 bids was treated by placing one tablespoon of 1.0% malathion dust in the nests. The eggs and the young squabs were lifted out while the dust was applied to the nests and then they were returned immediately.

At each ranch several heavily infested birds were maintained as a check for 30 days. Twenty-five per cent of the birds were examined for lice each week for 120 days after treatment.

RESULTS.—The results of this study are shown in table 1; each of the treatments gave complete control of the lice for more than 4 months. Putting the dust in the nest seems preferable because the work is easier and disturbance of the flock is less than that of direct application to individual birds.

Table 1.—Average number of each of two species of lice per pigeon following treatment with 4% malathion dust.

	Days After Treatment							
	Slender Pigeon Louse				Small Pigeon Louse			
	Before	7	30	120	Before	7	30	120
	Ranch A							
1.0% Dust[a]	43	0	0	0	41	0	0	0
Untreated	127	30	100[b]	—	35	68	125[b]	—
	Ranch B							
0.5% Dust[a]	33	0	0	0	26	0	0	0
1.0% Dust[c]	68	0	0	0	69	0	0	0
Untreated	75	37	61[b]	—	26	30	28[b]	—

[a] Applied by hand to individual birds.
[b] Discontinued after 30 days at the request of the grower.
[c] Applied to the nest only.

Observation showed that neither the careful temporary removal of the contents of the nest nor the malathion dust applied adversely affected (1) the hatchability of the eggs, (2) the newly hatched or growing squabs, or (3) the acceptability of the nest and the brooding responsibilities of the adult pigeons.

REFERENCE CITED

Lee, Alfred R., and Sheppard K. Haynes. 1915. Squab raising. U. S. Dept. Agric. Farmers' Bull. 801. Revised 1946.

[1] Accepted for publication April 6, 1959.

Parathion on Cover Crop and Fallowing for Control of Southern Potato Wireworm[1]

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The southern potato wireworm, *Conoderus falli* Lane (previously known as *C. vagus* Candeze), has been reported to be an important pest of Irish potatoes in northern Florida (Dobrovsky 1953) and coastal South Carolina (Deen & Cuthbert 1955).

Late fall and winter surveys in cultivated land in Charleston County, S. C., between 1955 and 1957 disclosed that larval populations of that insect were consistently low in fields in which summer-fall cover crops had been treated with parathion for control of foliage-feeding insects. They also were low in fields that had been planted to fall vegetable crops. As other studies revealed that the larvae responsible for most of the damage to tubers of spring crops of potatoes in that area hatch from eggs laid between late August and early October of the preceding year, the information obtained from the surveys suggested the possibility of controlling the insect by the following practices: (1) Applying parathion to the summer-fall crover crop to kill the adults before their eggs are laid or the newly-hatched fall-brood larvae before they leave the soil surface; (2) keeping the soil fallow during the fall egg-laying period, which is essentially what is done when it is prepared for a fall vegetable crop. An experiment designed to explore these possibilities was conducted near Charleston during 1958.

METHODS.—Five parathion treatments and the fallow treatment were compared with a check in six randomized blocks. The fallowed plots were 40 by 75 feet, all others 20 by 75 feet. The fallowed plots were kept practically free of vegetation by being disked or harrowed seven times at 10- to 17-day intervals be-

[1] Accepted for publication April 10, 1959.
[2] In cooperation with the South Carolina Agricultural Experiment Station.

Table 1.—Value of applications of parathion to cover crops at 2 pounds per acre and of fallowing the soil in reducing the fall brood of the southern potato wireworm, Charleston, S. C., 1958–59.

TREATMENT	NOVEMBER 7		DECEMBER 5		JANUARY 5	
	Larvae per Square Foot	Per Cent Control	Larvae per Square Foot	Per Cent Control	Larvae per Square Foot	Per Cent Control
Parathion spray, Sept. 9	0.2	98	—	—	—	—
Oct. 9	1.3	90	1.5	89	—	—
Nov. 10	—	—	2.8	80	2.0	78
Parathion granules, Sept. 9	0	100	—	—	—	—
Oct. 9	1.8	86	0.2	98	—	—
Fallowing	2.8	79	—	—	—	—
Untreated (vegetated)	13.3	—	14.0	—	9.2	—
L.S.D.[a] at 5% level	1.37	—	1.68	—	—	—
at 1% level	1.88	—	2.42	—	—	—

[a] Data for untreated plots not included.

tween July 15 and September 23. Other plots were allowed to grow up in native grasses and weeds, a practice found to be conducive to high populations of this wireworm. Parathion was applied at 2 pounds per acre, in sprays or granules. Emulsion sprays were broadcast at 100 gallons per acre with a tractor-mounted hydraulic sprayer operated at a pressure of 80 pounds per square inch and a tractor speed of 2 miles per hour. Five-per cent parathion granules were broadcast with a rotary hand duster. The vegetation was left undisturbed after the applications, and the insecticide was not mixed into the soil.

Results were based on numbers of wireworms sifted from 1 square foot of soil to a depth of 6 inches (four 6-inch cube subsamples) taken from the inner portion of each plot at various intervals after the application.

RESULTS.—The data are summarized in table 1. Treating the summer-fall cover crop with parathion appears to have considerable promise as a means of controlling the southern potato wireworm in land to be planted to early spring Irish potatoes in coastal South Carolina and possibly elsewhere in the Southeast. Such applications might also be of value in the control of insect pests of the cover crop. Applications made early in the oviposition period were of most value against wireworms. When applied near the end of that period, granules proved to be more effective then a spray, probably because a higher proportion of the insecticide reached the soil and the granules had longer residual effects.

Dead wireworm adults were found in the parathion-treated plots shortly after the September applications, indicating they killed many, if not most, of the females before eggs could be laid. It was apparent, however, that each of the applications was surprisingly effective against the larvae, even though the insecticide was not mixed into the soil. Until disturbed by plowing, southern potato wireworm larvae usually remain at or near the soil surface and therefore are vulnerable to a parathion application of the type just described.

Field plots kept practically free of vegetation during the egg-laying period had 79% fewer larvae by November 7 than unfallowed plots, but the population of 2.8 larvae per square foot then present in the fallowed plots is considered a potentially injurious one. It is possible that the plots used in the experiments were not large enough to show the full effect of fallowing. Eggs may have been laid in these plots by adults that found concealment in the nearby vegetated ones. Although apparently of some value in the control of the southern potato wireworm, late-summer and fall fallowing of land to be planted to spring-crop potatoes is undesirable from a horticultural standpoint, chiefly because it depletes the organic matter in the soil.

REFERENCES CITED

Deen, O. T., and F. P. Cuthbert, Jr. 1955. The distribution and relative abundance of wireworms in potato-growing areas of the Southeastern States. Jour. Econ. Ent. 48(2): 191–3.

Dobrovsky, T. M. 1953. Another wireworm of Irish potatoes. Jour. Econ. Ent. 46(6): 1115.

A Simple Rearing Cage[1]

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An inexpensive, rapidly assembled, and easily cleaned insect cage can be made from a pair of aluminum pans, wire screening, hardware-cloth, S-hooks, and rubber bands as shown in figure 1.

Cages 9, 12, and 14 inches in diamter can be made with readily available household utensils. Cages 10 to 16 inches long have proved to be very satisfactory, but the length can be varied to meet individual requirements.

The basic unit is a cylinder of wire screen the same diameter as the pans used as closures. Tight fits between wire and pans will enhance stability and prevent escape of small insects. The cage is held together by pairs of large rubber bands stretched between ¾-inch S-hooks slipped over the rims of the pans. A food-holding or resting platform of hardware-cloth can be placed within the cylinder before it is closed.

A 9-inch diameter cage can be made from a pair of cake pans,[2] one of which has a removeable bottom, a 12-inch diameter cage from a juice catcher pan[3] and a pizza pan,[4] and a 14-inch diameter cage from a pair of pizza pans.[5] It will be necessary to cut a hole in one of the 14-inch pizza pans to make an opening for this size cage.

To obtain a satisfactory cylinder, the screen wire should be cut about 32 inches for 9-inch diameter cages, 43 inches for 12-inch diameter cages, and 50 inches for 14-inch diameter cages. This allows an overlap sufficient to give a tight fit along the length of the seam. It is necessary to fasten the screen with wire paper-staples at the ends of the cylinder only.

For most insects, small strips of masking tape will suffice to hold the lose bottom of the cake pan in place. The hole in the juice catcher pan can be closed by using a 5-inch square of screen with a wooden tongue depressor stapled in its center. The lip of the hole places sufficient tension on the wire to make a tight fit when the depressor is slipped inside.

When the cage becomes fouled, it can be taken apart, the screen discarded, the pans washed or sterilized, and a fresh cage rapidly reassembled.

In addition to rearing, the cages could be used in the green-

[1] Accepted for publication April 10, 1959.

[2] *Mirro* Nos. 1169M and 1179M, 9×1½ inches at $0.48 and $0.64 in lots of six, respectively.

[3] *Mirro* No. 8412L, at $0.29 in dozen lots.

[4] *Mirro* No. 322M, at $0.52 in lots of four.

[5] *Wear-Ever* No. 2774.

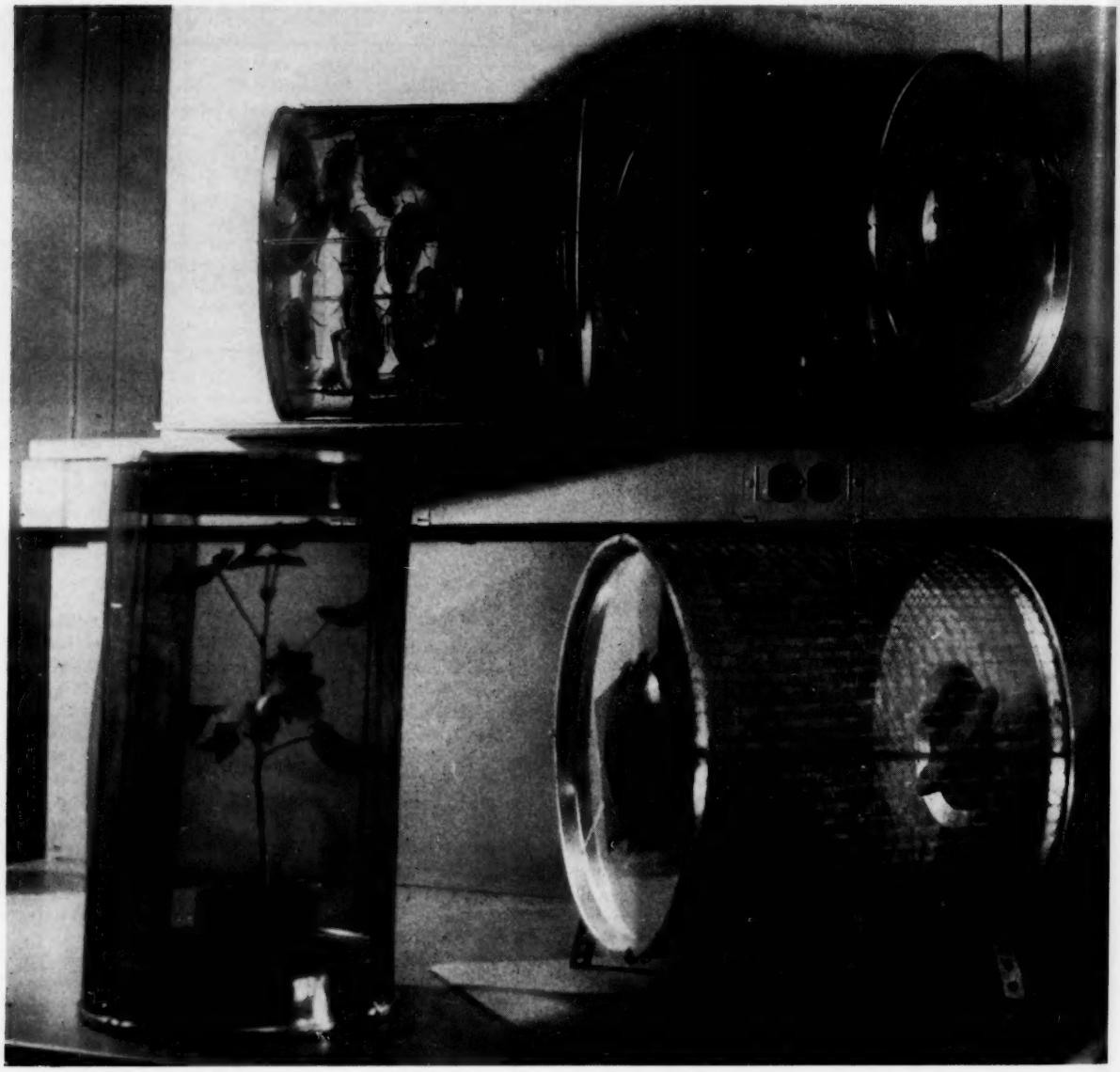

FIG. 1—Insect and animal cages.

house or field to house insects on potted or growing plants, to protect selected plants from insect attack, or to trap insects emerging from the soil.

"Self-cleaning" cages for mice, rats, guinea pigs and other small animals can be made by using hardware-cloth of appropriate mesh for the cylinder instead of wire screening. Here it would perhaps be best to use curtain-rod type springs rather than rubber bands to hold the cage together.

Malathion Dust for the Control of Lice on Domestic Game Birds[1]

JOHN L. RODRIGUEZ and L. A. RIEHL,[2] *University of California Citrus Experiment Station, Riverside*

In southern California, game bird breeding is a relatively small segment of the poultry industry. However, expansion is encouraged by high prices for the meat, breeding stock, and hatching eggs.

The high population density used for game birds in rearing pens favors infestations and makes it extremely difficult to examine birds periodically. Ectoparasite infestations can cause serious stress to the vitality of these semiwild birds. The premium aspects of the commodity place a high economic importance on any deleterious factors.

Little or no studies have been made of pest control of domestic game birds. Growers tend to neglect pest control or apply treatments recommended for chickens. Both practices are unsatisfactory and a definite need for pest control investigation was demonstrated.

The known efficacy against lice of several species of poultry and safety of the treatment indicated malathion dust as the insecticide of choice for this experiment.

[1] Accepted for publication April 15, 1959.

[2] The authors wish to express their sincere appreciation to Dr. P. T. Johnson of the U.S.D.A. Agric. Res. Service Insect Identification Laboratory, Plant Industry Station, Beltsville, Maryland for the identification of the game bird lice.

MATERIALS AND METHODS.—A ranch near Norco, California reported a louse infestation on several species of game birds. Examination of random birds showed Chukar partridge (*Alectoris graeca* Meisner), Bobwhite quail (*Colinus virginianus* Linnaeus), California quail (*Lophortyx californicus* Shaw), Golden pheasant (*Chrysolophus pictus*), Mongolian pheasant (*Phasianus colchicus mongolicus* Linnaeus), and Ringnecked pheasant (*Phasianus colchicus* Linnaeus) heavily infested with wing lice (*Lipeurus* sp.) and two varieties of body lice (*Goniocotes* sp. and *Goniodes* sp.) of the family Philopteridae. In addition, large numbers of eggs of lice were found on the wings and bodies of the infested birds. Several other species of game birds on the ranch were examined and were found free of lice.

All the birds were kept in pens with the ground as the floor. Small wooden shelters were distributed at random in the enclosures.

In each pen, generally in a sheltered spot, there were small natural dusting wallows. Therefore, applications of the insecticide to the wallows for self treatment by the birds appeared to be the most practical approach to control of lice. All the wallows, in each of nine pens containing infested game birds, were dusted liberally with 1% malathion dust. The average amount of dust used per pen was 1 pound. A small pen, containing three Chukar partridges infested with lice was used as the control and these birds were treated after 30 days in order to comply with the wishes of the grower.

A total of 64 Chukar partridges, 325 California quail, 150 Bobwhite quail, 23 Golden, 15 Mangolian, and 55 Ringnecked pheasants were treated.

The handling of individual birds to count the lice involved considerable risk of injury because they were easily excitable and flew into fences, etc., or crowded into piles in corners. In addition, the birds that were examined lost a great many feathers and there was a serious risk of inducing molt, which would possibly cause lowered egg production. Accordingly, the handling was reduced to a minimum and only 2 to 5% of the birds were examined at each interval.

The malathion dust was not placed near water or feed and the birds were not unduly excited during application of the insecticide to the wallows. No reduction in egg production or consumption of feed or water, or symptoms of toxicity were apparent following application of the treatment.

DISCUSSION OF RESULTS.—Results of this study are shown in table 1. Seven days after treatment, the single malathion application to the wallows gave complete control of the three varieties of lice. The birds remained free of lice for more than 90 days. Putting the malathion in the dust-bath wallows seems to be the most inexpensive and simple method of treating the semiwild birds. There is little or no disturbance to the flock, no loss in egg production nor in hatchability of the eggs.

Table 1.—Average number of wing and body lice on several species of game birds at intervals after application of 1% malathion dust to the natural dust-bath wallows of the pens.

GAME BIRD	DAYS AFTER TREATMENT									
	Wing Lice					Body Lice				
	0	7	14	30	90	0	7	14	30	90
	Treated									
Partridge	56	0	0	0	0	21	0	0	0	0
Quail	11	0	0	0	0	38	0	0	0	0
Pheasant	102	0	0	0	0	19	0	0	0	0
	Untreated[a]									
Partridge	100	78	91	72	—	25	31	19	21	—

[a] Treated after 30 days at the request of the grower.

A Semiautomatic Liquid Fly Bait Dispenser[1]

JOHN W. KILPATRICK and H. F. SCHOOF[2]

With renewal at frequent intervals, organophosphorus poison baits will effectively control house fly (*Musca domestica* L.) populations at many problem sites. The period between successive treatments depends on the densities prevailing, as well as local environmental factors (*e.g.*, dust, litter, rain). Since a chief disadvantage of these baits is the need for periodic replenishment, workers have attempted to establish bait stations of a semipermanent nature (Keller *et al.* 1956, Kilpatrick & Schoof 1958). This note describes a residual technique involving liquid baits.

The bait dispenser employed consisted of a 1-gallon chicken watering device which maintained a constant level of liquid in the base trough (fig. 1). The toxicant, either DDVP or Dipterex®(*O,O*-dimethyl, 2,2,2-trichlor-1-hydroxyethylphosphonate), was dissolved in water at concentrations of 0.1 and 1.0%, respectively. Sugar was added to each formulation at a rate of 1 pound per gallon (12.5%). After preliminary tests indicated that dead flies rapidly clogged the dispenser, a screen wire barrier was installed over the base trough. This screen allowed the flies to feed, but prevented their accumulation in the trough. The number of dispensers used at a problem site varied with the fly population and area involved.

At dairy "H," 11 dispensers were used: 6 in the milking barn, 2 in the feed barn, and 3 in calf pens. Prior to treatment with DDVP in mid-June, fly indices for the 2 preceding weeks were 86 and 177. In the first posttreatment week, the index dropped to 43; for posttreatment weeks 2 through 7, the respective indices averaged 14, 8, 5, 7, 5, and 3 flies. In the eighth week (mid-August) control was lost, when the index rose to 74 flies. After the dispensers were cleaned and recharged with fresh bait, fly indices dropped to 10 and 19 specimens for the next 2 weeks. However, in the third and fourth weeks, counts increased to 58 and 177 flies, this loss of control apparently being caused by the overturning of a number of the dispensers by dairy personnel. Therefore, all dispensers were cleaned in early September and recharged with Dipterex. One week after installation, the fly index dropped to 10 flies; levels remained between 7 and 13 flies for the next 4 weeks.

At chicken Ranch "R," 11 bait dispensers were distributed among the chicken batteries, and 6 in the adjacent hog barn. For evaluation purposes, the indices in the chicken ranch *per se* were the only counts considered. After treatment in mid-June with DDVP, fly indices during the first 2 weeks showed little change from pretreatment levels of 55 and 47. However, in the third and fourth posttreatment weeks, counts dropped to 15 and 22 flies respectively, this increased efficiency being considered the result of relocation of the dispenser. Initially, the units in the chicken batteries were so situated that chicken droppings and feathers clogged the troughs. Cleaning the dispensers and replenishing the DDVP baits in mid-August resulted in the lowering of fly indices to levels of 6, 7, 12, 4, 9, and 19 flies for the ensuing 6 weeks. On the seventh and eighth weeks, indices rose to 26 and 47 flies.

The technique utilizing the chicken watering device offers promise as a means of dispensing liquid fly baits for extended periods (6 weeks) without the necessity of retreatment. The only care required is for the operator to twirl the screen ring weekly so as to dislodge the dead flies thereon. This action prevents accumulation of flies on the screens and allows the dispenser to operate at its maximum efficiency.

[1] Accepted for publication April 17, 1959.

[2] From the Communicable Disease Center, Bureau of State Service, Public Health Service, U.S. Department of Health, Education, and Welfare, Savannah, Georgia.

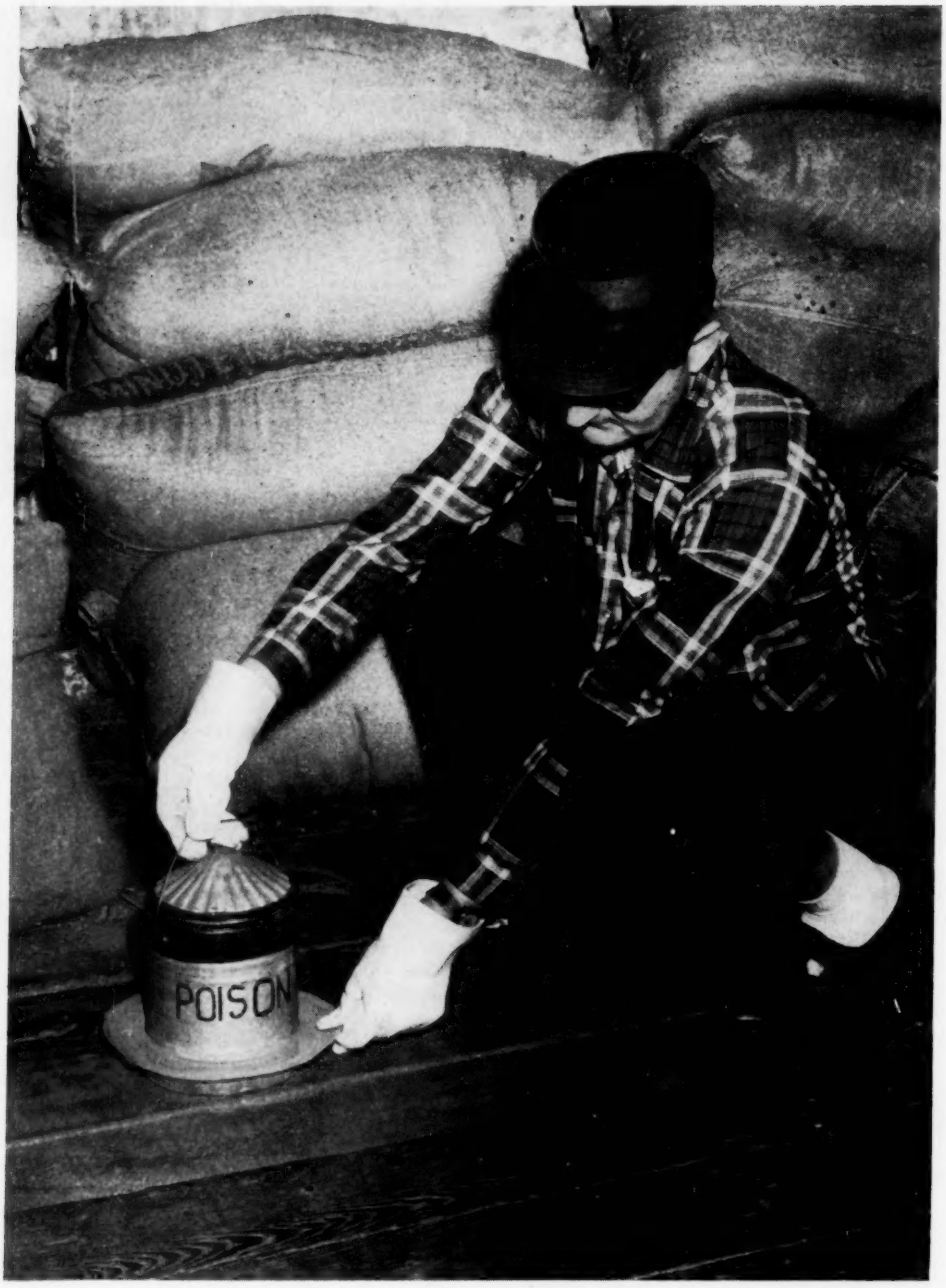

FIG. 1—Semiautomatic bait dispenser.

REFERENCES CITED

Keller, J. C., H. G. Wilson, and C. N. Smith. 1956. Bait stations for the control of house flies. Jour. Econ. Ent. 49(6): 751–2.

Kilpatrick, J. W., and H. F. Schoof. 1958. A field strain of malathion-resistant house flies. Jour. Econ. Ent. 51(1): 18–19.

Dilan Formulations for Adult House Fly Control[1]

H. F. SCHOOF and JOHN W. KILPATRICK[2]

In previously reported tests (Kilpatrick & Schoof 1954), it was shown that Dilan[3]:DDT:cottonseed oil and Dilan:cottonseed oil formulations gave 8 weeks of effective fly control in two

[1] Accepted for publication April 17, 1959.

[2] From the Communicable Disease Center, Bureau of State Service, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah Georgia.

[3] 1 part 1,1-bis(*p*-chlorphenyl)-2-nitropropane and 2 parts 1,1-bis(*p*-chlorophenyl)-2-nitrobutane.

Table 1.—Effectiveness of Dilan residual applications against *Musca domestica* in dairies.[a]

WEEKS AFTER TREATMENT	DAIRY AND GRILL INDICES (3-WEEK MOVING AVERAGE)			
	"Rg"[b]	"T 1"[c]	"H"[d]	"KM"[c]
−2	185	352	838	590
−1	207	347	937	637
1	44	13	17	64
2	34	34	25	60
3	48	58	25	34
4	101	61	23	13
5	160	69	27	12
6	—	72	34	14
7	—	84	51	14
8	—	121	99	13
9	—	—	125	42
10	—	—	162	88

[a] Treated in late May.
[b] Dilan: DDT.
[c] Dilan: cottonseed oil.
[d] Dilan: DDT: cottonseed oil.
[e] Dilan.

dairies. Since both treatments were applied in late summer further tests were scheduled for the following spring to evaluate the pesticide under conditions more suitable for maximum fly production.

Five dairies were treated in late May as follows: dairies "Ba" and "KM," Dilan (200 mg./sq. ft.); dairy "Rg," Dilan:DDT (200:200 mg./sq.ft.); dairy "H," Dilan:DDT:cottonseed oil (200:200:80 mg./sq.ft.); and dairy "T-1," Dilan:cottonseed oil (200:80 mg./sq.ft.). All applications were at 100 p.s.i. pressure with a power sprayer equipped with a flat spray nozzle.

Within 1 week after treatment, fly grill indices were reduced markedly at four dairies (table 1). The Dilan:DDT:cottonseed oil applications gave satisfactory control (20 flies/grill count or less) the initial posttreatment week, and indices slightly above that level for the next 4 weeks. The Dilan:cottonseed oil applications also gave only 1 week of control, while the Dilan:DDT treatment failed to reduce indices to a satisfactory level.

At dairy "KM," the Dilan residues alone produced a continuing decline in fly densities, but the latter did not reach a satisfactory control level until the fourth posttreatment week. For the following 5 weeks satisfactory indices prevailed, after which densities rose rapidly.

The sustained low fly levels at dairy "KM" was puzzling in view of unsatisfactory laboratory results obtained with this formulation. Consequently, the same treatment was applied at dairy "Ba," where it initially reduced densities from a level of 498 to 24 flies/grill count. However, within 2 weeks, the grill index increased to 240 flies.

The failure of the Dilan:DDT:cottonseed oil and Dilan:cottonseed oil formulations to give results comparable to those obtained the previous year (Kilpatrick & Schoof 1954) is attributed to an effect of rising versus declining seasonal fly populations. Thus, these data, together with previous laboratory and field findings, indicate that the potential of Dilan as an effective residual treatment is limited chiefly to the Dilan:DDT:cottonseed oil formulation.

REFERENCE CITED

Kilpatrick, J. W., and H. F. Schoof. 1954. House fly control in dairies near Savannah, Ga., with residual applications of CS-708, NPD, and malathion. Jour. Econ. Ent. 47(6): 991–1001.

Effectiveness of New Insecticides against the Harlequin Cabbage Bug on Collards[1]

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Occasionally the harlequin cabbage bug, *Murgantia histrionica* (Hahn), becomes a severe pest of kale, collards, cabbage, turnip greens, broccoli, and other cole crops in southeastern Virginia. In the past, growers have experienced considerable difficulty in controlling this insect, especially in the adult stage. Lately, numerous complaints have been received concerning the lack of effectiveness of 20% sabadilla dust, the recommended insecticide.

Gaines & Dean (1948) reported the results of field tests with dusts of DDT, toxaphene, and 20% sabadilla and found that, while these chemicals gave good control of the harlequin cabbage bug nymphs, sabadilla and DDT were less effective against the adults.

The investigations reported in this paper were undertaken as part of an extensive program to improve the control of cole crop pests in southeastern Virginia.

MATERIALS AND PROCEDURE.—Five experiments involving 21 insecticides and 49 different treatments were conducted at Norfolk, Virginia, in 1957. All insecticides were tested as sprays in the emulsion form except Dylox® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), (soluble powder) and sabadilla (20% dust). The test materials, with the exception of sabadilla, were applied to the Vates variety of collards at the rate of 100 gallons of water per acre by means of a knapsack sprayer operating at 40 pounds pressure. A rotary hand duster was used in applying the sabadilla dust. The collard plots consisted of 5 rows, each 30 feet long, with 3 untreated rows between each plot. All treatments were replicated four times in randomized blocks.

In evaluating the relative effectiveness of the insecticides, both harlequin cabbage bug adults and last instar nymphs were observed. Previous to treating, 50 last instar nymphs were carefully counted on the collard plants in a section of each plot and that part of the row, usually less than 10 feet, isolated from adjacent plants insofar as possible. Adult harlequin cabbage bugs were collected from this same general area but were transferred to another planting of smaller uninfested collards. Ten adults were placed on each of two plants in each replication. As soon as these plants were treated, a cylindrical wire screen cage 16″×12″ was quickly placed overall. Although only a small part of each plot held test insects, the entire section was treated in order to insure a more uniform coverage.

RESULTS AND DISCUSSION.—Results given in table 1 show that numerous insecticides were very effective against both nymphs and adults of the harlequin cabbage bug. Sevin® (1-naphthyl-*N*-methyl carbamate) and Thiodan® (6,7,8,9,10,10=hexachloro-1,5,5*a*,6,9,9*a*,hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) were especially rapid in action, giving 100% knockdown in 6 hours or less. Other insecticides, such as Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) and Dylox were slower in action but eventually gave excellent control.

Statistical analysis indicated that DDT, Perthane® (1,1-dichloro-2,2-bis(p-ethylphenyl)ethane), 20% sabadilla, and Union Carbide Chemicals Compound 8305 (P-chloro-2,4-dioxa-5-methyl-P-thiono-3-phosphabicyclo (4.4.0) decane) were significantly less effective than the other insecticides against adult harlequin bugs. Extensive feeding damage also occurred in these treatments. Last instar nymphs were definitely easier to control than the adults but in general the order of relative insecticidal effectiveness was the same.

[1] Contribution from the Entomology Department, Virginia Truck Experiment Station. Paper No. 131, Journal Series. Approved for publication April 13, 1959. Accepted for publication April 17, 1959.

Table 1.—Effectiveness of new insecticides against the harlequin cabbage bug on collards.

Treatment and Pounds of Actual Toxicant per Acre	Per Cent Harlequin Bugs Down at Hours Indicated					
	6		24		72	
	Adults	Nymphs	Adults	Nymphs	Adults	Nymphs
Experiment 1 (Treated Sept. 2, 1957, Norfolk, Va.)						
DDT, 0.5	4	16	28	57	75	87
Demeton, 0.5	58	61	100	100	100	100
Diazinon®,[a] 0.5	13	31	80	95	98	100
Endrin, 0.5	63	70	100	100	100	100
Ethion, 0.5	18	17	44	77	93	96
Perthane, 0.5	0	0	40	52	63	70
Sabadilla, 5.0	13	13	34	61	79	92
Sevin, 1.0	100	100	100	100	100	100
Thimet®,[b] 0.5	41	65	100	100	100	100
Untreated	0	2	0	2	0	2
Experiment 2 (Treated Sept. 5, 1957, Norfolk, Va.)						
DDT, 0.5	6	23	18	62	78	88
Phosdrin®,[c] 0.25	65	100	95	100	96	100
Phosdrin, 0.5	95	100	100	100	100	100
Parathion, 0.25	41	81	80	92	93	99
Sabadilla, 5.0	8	25	49	74	81	91
Sevin, 0.5	100	100	100	100	100	100
Sevin, 1.0	100	100	100	100	100	100
Toxaphene, 2.0	20	59	58	72	89	95
CPD 8305, 0.5	5	72	64	81	81	93
Untreated	0	4	0	4	0	4
Experiment 3 (Treated Sept. 25, 1957, Norfolk, Va.)						
Dibrom, 0.5	51	75	100	100	100	100
Dylox, 1.0	16	21	75	93	100	100
Parathion, 0.25 +toxaphene, 2.0	67	95	93	100	100	100
Sevin, 0.5	100	100	100	100	100	100
Thiodan, 0.5	100	100	100	100	100	100
Trithion, 0.5	9	30	74	83	100	100
Untreated	3	0	3	3	3	4

[a] *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.
[b] *O,O*-diethyl *S*-ethylthio)methyl phosphorodithioate.
[c] 1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical.

A combination of parathion and toxaphene produced better results than either of these chemicals applied separately. The response of the harlequin bug to the parathion-toxaphene formulation is of interest since this treatment is widely used against cole crop pests in southeastern Virginia. Observations in commercial plantings of broccoli and cabbage where the growers were on a 7-day-treatment schedule indicated that parathion-toxaphene completely controlled the harlequin bug.

Some seasonal variation was noted. In an earlier test, Dibrom® (*O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate) gave only an 85% kill of harlequin cabbage bug adults on collards as compared with 100% in Experiment 3. Also in this test, the parathion-toxaphene combination failed to give the complete control previously reported. The poorer results were associated with hot, dry weather and a low relative humidity. Gaines & Dean (1948) suggested that a low relative humidity may have been responsible for some of the poor control obtained in their tests against the harlequin cabbage bug in Texas.

Reference Cited

Gaines, J. C., and H. A. Dean. 1948. Comparison of insecticides for control of Harlequin bugs. Jour. Econ. Ent. 41(5): 808–9.

Systemic Insecticides for the Control of the Black Cherry Aphid[1]

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In the Willamette Valley of Oregon the black cherry aphid, *Myzus cerasi* (Fab.), causes serious injury to cherry trees by stunting or killing terminal growth and secreting honey-dew, which causes the cherries to be sticky and difficult to harvest. Furthermore, aphid honey-dew may create a contamination problem in the brining of cherries by causing the aphids to stick to the cherries and to the brining vats.

Dormant spraying with DN compounds for the control of aphid eggs and the use of nonsystemic organic phosphate insecticides and nicotine compounds have only partially controlled this aphid. Reinfestation occurs in late May or June by migrating winged aphids.

Table 1.—Black cherry aphid infestation 15 days after cherry trees were treated with systemic insecticides, 1954.

Insecticide	Average Aphids per Replicate	
	Trees Sprayed	Tree Trunks Painted
Demeton 26.2% E.C. 1-1600	0	0
Demeton 26.2% E.C. 1-800	0	—
Demeton 13.1% E.C.	—	0
Am. Cyanamid 12008 48% E.C. 1-1600	0	0
Am. Cyanamid 12008 48% E.C. 1-800	0	—
Am. Cyanamid 12008 24% E.C.	—	0
Am. Cyanamid 12009 48% E.C. 1-1600	41	6
Am. Cyanamid 12009 48% E.C. 1-800	16	—
Am. Cyanamid 12009 24% E.C.	—	7
None	149.2	149.2

Experimental tests for the control of the black cherry aphid with systemic insecticides were initiated on July 1, 1954. The initial testing included spraying 3-year-old cherry trees with diluted systemic insecticides and painting the trunks of other 3-year-old cherry trees with concentrated and semiconcentrated systemic compounds. Single tree plots were randomized and replicated three times for each material and concentration of the insecticide tested. Results were evaluated by counting the live aphids on 10 infested leaves from each tree. Four untreated trees were left as checks.

All the systemic insecticides tested were emulsifiable concentrates. The concentration for each systemic insecticide tested as a spray was as follows: demeton 26.2%, 1–1600 and 1–800; Am. Cyanamid 12008 (*O,O*,diethyl *S*-(isopropylthio)methyl phosphorodithioate and Am. Cyanamid Company's formulation 12009 (*O,O*-diethyl *S*-propylthio)methyl phosphorodithioate), 48%, at concentrations of 1–1600 and 1–800.

The concentrations of the insecticides painted on the tree trunks were as follows: demeton 26.2% and 13.1%; Am. Cyanamid 12008 and 12009, 48% and 24%. The tree trunks were treated from the ground level to approximately 1 foot above the ground.

Aphid counts were made 15 days after the trees were treated. No aphids survived the treatments on either the sprayed or trunk-treated trees where demeton or compound 12008 was used. Live aphids were found on trees sprayed and trunk-treated with insecticide 12009. Table 1 shows the relative effectiveness of the insecticides used in the tests.

All the trees that were trunk-treated showed discoloration and flecking of the bark near the cambium layer. No evidence of injury appeared on the sprayed trees.

In 1956, 1957, and 1958 systemic insecticides were tested as pre-bloom and petal-fall sprays on bearing cherry trees for the control of the black cherry aphid. In 1956 Mazzard seedling trees were used for testing demeton 26.2% at a concentration of 1–800

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[2] Residue analysis was conducted by Ulo Kiigemagi, Agricultural Chemistry Department, Oregon State College.
[3] The author acknowledges the assistance of Kenneth Goeden and J. E. Gilmore, graduate assistants, in these studies.

Table 2.—Effectiveness of systemic insecticides against the black cherry aphid when applied as pre-blossom and petal-fall sprays, 1956, 1957, and 1958.

Orchard No.	Insecticide	Stage of development and date of application	Year	Date examined	Number of infested terminal tips
1	Demeton 26.2%, E.C. 1-800	Pre-blossom April 13	1956	June 23	0[a]
1	Check	—	1956	June 23	650
2	Demeton 26.2%, E.C. 1-800	Pre-blossom April 13	1956	June 23	0
2	Check	—	1956	June 23	25
2	Demeton 26.2%, E.C. 1-400	Pre-blossom April 8	1957	June 4	0
2	Thimet 48%, E.C. 1-400	Pre-blossom April 8	1957	June 4	0
2	Check 1	—	1957	June 4	70
2	Demeton 26.2%, E.C. 1-1600	Petal-fall April 30	1957	June 4	0
2	Check 2	—	1957	June 4	20
1	Demeton 26.2%, E.C. 1-800	Petal-fall April 30	1957	June 4	0
1	Check	—	1957	June 4	205
3	Demeton 26.2%, E.C. 1-800	Pre-blossom March 28	1958	June 18	0
3	Demeton 26.2%, E.C. 1-800 + Wettable sulfur (Kolofog) 6 lb.:100 gal.	Pre-blossom	1958	June 18	12
3	Check	—	1958	June 18	427

[a] One infested leaf found.

against the black cherry aphid as a pre-blossom spray. The tests were conducted in two small orchards which were widely separated. Orchard 1 included 16 trees of which 10 were sprayed and 6 were left as check trees. Orchard 2 included seven trees of which three were sprayed and four were left as checks.

The plots were sprayed on April 13 and the orchards were checked on June 23. The sprayed trees in orchard 1 showed one infested leaf compared with 650 infested terminal tips on the adjacent unsprayed trees. In orchard 2 no infested terminal tips appeared on the sprayed trees. The unsprayed trees showed 25 infested terminal tips.

In 1957 demeton and Thimet® (*O,O*-diethyl *S*-(ethylthio)-methyl phosphorodithioate were tested as pre-bloom sprays in two small cherry orchards. Commercial varieties of cherries and Mazzard seedlings were used as test trees. Both Thimet 48%, 1–400 and demeton 26.2%, 1–1600, 1–800, and 1–400, killed 100% of the aphids for the season when the applications were made immediately before bloom or in the petal-fall stage of fruit development. Adjacent check trees were infested. Table 2 gives the results of a comparison between demeton and Thimet and between the sprayed and unsprayed plots.

In 1958 demeton 26.2%, 1–800, was combined with wettable sulfur (Kolofog) 6 pounds to 100 gallons and compared with demeton 26.2%, 1–800 without a fungicide, against the black cherry aphid. These sprays were applied immediately before bloom on March 28. The experiment was conducted in a commercial orchard using mixed varieties of sweet cherries. Each plot contained approximately five trees and was replicated three times.

Terminal tips on the plots sprayed with demeton without a fungicide showed no infestation at any time during the season. Plots sprayed with demeton plus wettable sulfur showed a total of 12 infested terminal tips. In comparison the check plots showed 427 infested terminal tips (table 2).

Cherries from plots sprayed with demeton 26.2%, 1–800 and 1–400, were taken at the normal harvest time for residue analyses. The samples were extracted immediately after harvest and were analyzed within 2 weeks, using the modified enzymatic method of J. W. Cook (1954). No demeton residue was detected in the cherries.

Reference Cited

Cook, J. W. 1954. Report on the determination of insecticides by enzymatic methods. Jour. Assoc. Offic. Agric. Chemists. **37**(2): 561–4.

The Effectiveness of Ronnel as a Cord Impregnant for House Fly Control[1]

John W. Kilpatrick and H. F. Schoof[2]

Previous studies on the control of house flies (*Musca domestica* L.) by the use of impregnated cords showed that parathion and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) Kilpatrick & Schoof 1956, Schoof & Kilpatrick 1957) were the toxicants best suited for that purpose at that time. Ten per cent parathionxylene solutions were adequate to treat cotton cords (3/32″ diameter), but with Diazinon it was necessary to impregnate larger cords (3/16″ diameter) with a 25% Diazinon-xylene solution to obtain comparable control. Because of the extended effectiveness of cord treatments (9 to 24 weeks control), new toxicants are tested routinely as cord impregnants. This paper describes studies with the insecticide, ronnel (Dow ET-57 sampled as Dow ET-14).

All cords (3/32″ diameter) were impregnated with a 25% ronnel-xylene solution, the latter being prepared from technical material for eight of the test sites and from a 50% commercial formulation for two sites. The cords were installed in the dairies at a rate of 30 linear feet per 100 square feet of floor area during the period of June 2 to August 5. Evaluation of the effectiveness of the treatments was by means of weekly inspections before and after cord installation. A grill index for each barn was obtained by averaging the four highest counts recorded at four different stations.

The results at six dairies (table 1) indicate that satisfactory to excellent control was achieved for 8 to 16 weeks. Only 9 weeks of control were obtained at dairy "Rg." At dairy "T-1," the treatment remained effective through 16 weeks when cold

Table 1.—Grill indices (3-week moving average) at dairies treated with ronnel-impregnated cords.

Weeks after treatment	Dairies and grill index					
	"Bg"	"S"	"T-1"	"Ra"	"W"	"Rg"
−2	37	127	61	34	43	101
−1	55	121	100	60	58	119
1	1	9	12	11	2	21
2	1	5	10	9	3	17
3	1	3	6	7	3	10
4	2	3	6	7	3	7
5	2	3	4	6	2	7
6	3	4	4	5	2	7
7	2	6	5	3	2	6
8	2	5	6	4[a]	2	6
9	2	3	8		4	16
10	3	3[a]	7		6[a]	42
11	3[a]		4			63
12			4			50
13			4			
14			7			
15			10			
16			13			
Date treated	7-10-58	7-1-58	6-9-58	8-5-57	7-22-58	6-10-58

[a] Termination of tests.

weather terminated the tests. The 8- to 11-week period for dairies "Bg," "S," "Ra" and "W" also represents the maximum observation period possible due to the onset of cold weather.

The data for dairies "Km" and "Mo" (table 2) show satisfactory to excellent control for dairy "Mo" through week 9, and excellent control for 10 weeks at dairy "Km." All cords then were removed from both dairies and the dairies remained without treatment for the following 3 to 4 weeks. During that interval, grill indices climbed rapidly from less than 10 to over 300 flies per grill count. When counts reached those maximum levels,

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[2] From the Communicable Disease Center, Bureau of State Service, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia.

Table 2.—The effect of removal and/or reinstallation of ronnel-treated cords on weekly house fly indices at three dairies.

Weeks After Treatment	Dairies and Grill Index		
	"Ro"	"Mo"	"Km"
−2	81	91	191
−1	315	152	228
1	46	36	1
2	41	55	2
3	7	8	1
4	8	7	1
5	6	7	1
6	6	8	3
7	4	4	1
8	5	9	2
9	4	9[a]	2
10	3	54	2[a]
11	12	82	6
12	4[a]	359[b]	88
13	23	37	308
14	75	10	214[b]
15	48	16	15
16	36	20	32
Date treated	6-5-58	6-5-58	6-30-58

[a] Cord removed.
[b] Old cord reinstalled.

Table 3.—Weekly grill index at a dairy "VW" treated with ronnel (A) or parathion (B) impregnated cords.

Weeks After Treatment	Grill Index		
	A	A	B
−2	52	213	90
−1	150	331	237
1	30	159	40
2	86	131	124
3	213	90	228
4	331	237	196
			128
Date treated	6-6-58	7-2-58	7-31-58

reinstallation of the same cords caused the fly densities to return to reduced levels within 2 weeks. At dairy "Ro," removal of the cords after 12 weeks of control led to a moderate increase in fly densities.

At only one dairy, "VW," did the ronnel-treated cords fail to give satisfactory results (table 3). Installation of the cords at this dairy reduced the grill index from 150 to 30 flies but subsequent inspections showed rising densities. After the index reached 396 flies, the original cords were replaced with newly treated cords, but the latter had little effect upon the fly level. To determine if the results reflected the inadequacy of the toxicant employed, parathion-treated cords were substituted for those treated with ronnel, but these also failed to provide satisfactory control. Inasmuch as the fly potential at this dairy was not exceptionally high, no obvious explanation arises for the poor results obtained there. Unfortunately, efforts to study the problem further were thwarted by the sale of the dairy lands for a housing development.

Based on the results to date, the ronnel-treated cords offer more promise for extended control than the same toxicant used as a residual spray application since the latter provided a maximum control period of 8 to 9 weeks when used at a rate of 200 mg./sq. ft. (Kilpatrick & Schoof 1958). Ronnel is the first organophosphorus compound of relatively low mammalian toxicity that has been effective as a cord impregnant for house fly control. The extended control achieved with ronnel-impregnated cords compares favorably with that obtained previously with parathion- or Diazinon-impregnated cords.

References Cited

Kilpatrick, J. W., and H. F. Schoof. 1956. The use of insecticide treated cords for house fly control. Publ. Hlth. Rpts. **71**(2): 144–59.

Kilpatrick, J. W., and H. F. Schoof. 1958. House fly control studies in Chatham County, Georgia. Jour. Econ. Ent. **51**(6): 908–10.

Schoof, H. F., and John W. Kilpatrick. 1957. House fly control with parathion and Diazinon impregnated cords in dairy barns and dining halls. Jour. Econ. Ent. **50**(1): 24–7.

Evaluation of Certain Insecticides for Southern Potato Wireworm Control[1]

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The southern potato wireworm, *Conoderus falli* Lane, which was reported by Deen & Cuthbert (1955) to be an important pest of potatoes in the Southeast, became resistant by 1956 in Charleston County, S. C., to the previously effective insecticides chlordane, dieldrin, heptachlor, and aldrin (Reid & Cuthbert 1956). Similar resistance was reported by Norris (1957) in potato-producing areas near Hastings, Fla., where Dobrovsky (1953) previously reported the insect as being a pest of potatoes. Efforts to find other effective insecticides were made by the writers in the vicinity of Charleston, S. C., during 1957 and 1958.

DDT, at a dosage of 20 pounds per acre, had proven less effective in earlier tests than the materials just mentioned, but was given more trials and used extensively by commercial potato growers in Charleston County in 1957. In general, the control provided by DDT that year was satisfactory, but erratic results were obtained, especially in soils high in organic matter.

Laboratory screening tests in 1957 disclosed that parathion was toxic to southern potato wireworms at a dosage as low as the equivalent of one-half pound per acre. From 1 to 4 pounds per acre gave good control in small-scale field tests in the fall and early winter of 1957, and 2 and 4 pounds provided adequate protection to experimental and commercial plantings of potatoes in the spring of 1958. Critical evaluation of the toxicity of parathion to the insect under field conditions was not possible at that time, because relatively low larval populations survived the winter. No detectable residues were found on tubers grown in soil treated with 2, 4, or 8 pounds per acre shortly before the crop was planted. The residual effectiveness of parathion in the soil was found to be relatively short, about 5 weeks for the 2-pound dosage. This short residual action is a disadvantage, as some larvae are not contacted during this period.

This paper reports a field-plot comparison of parathion with several other insecticides selected as having most promise for southern potato wireworm control from 73 compounds tested in a laboratory screening program.

Each compound was tested in five randomized-block replications of 9- by 53-foot plots. Parathion, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), Shell

[1] Accepted for publication April 21, 1959.
[2] In cooperation with the South Carolina Agricultural Experiment Station.

SD 4402 (1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan), Am. Cyanamid 18,133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate), and ENT-21,170 (2,4-dimethylbenzyl chrysanthemumate) were applied to the soil surface in emulsion sprays at 100 gallons per acre with a tractor-mounted hydraulic sprayer. Thimet® (*O,O*-diethyl *S*-(ethylthiomethyl) phosphorodithioate) was applied in 2% granules with a rotary hand duster, and Nemagon® (dibromo-chloropropane) in 10% granules broadcast by hand. These materials were mixed into the upper 4 inches of soil within 1½ hours after their application by running a disk harrow once over the middle 6- by 53-foot portion of each plot. D-D mixture (1,2-dichloropropane and 1,3-dichloropropene) and ethylene dibromide were applied by pouring the liquid into holes punched 6 inches deep on 12-inch centers in the soil. The holes were immediately filled with soil. Ethylene dibromide was applied on October 31, 1958, and all other materials on the preceding day.

Soil used in cage tests to determine residual effectiveness of certain materials was obtained from the plots by taking thirty ¾-inch cores 4 inches deep from each of two replicates. This soil was screened and a quart of each lot saved for the tests. After being mixed for 15 minutes in an electrical pebble mill, 40 ml. of the soil was placed in each of twenty 3-ounce salve cans. One field-collected wireworm was caged in each can and a grain of boiled corn provided as food. The cages were examined a week later and every 2 weeks thereafter.

The control obtained in the field plots was determined by screening 1 square foot of soil to the 6-inch depth (four 6- by 6- by 6-inch subsamples) from the center portion of each plot 33 days after the application. The residual effectiveness was based on the mortality of caged larvae 9 weeks after being placed in the treated soil 36 and 83 days after the application.

The data obtained are summarized in table 1. Am. Cyanamid 18,133, Thimet, SD 4402, and ethylene dibromide were as effective as parathion and gave adequate control of the wireworm population in field plots. Diazinon tended to be inferior to these compounds, but was not significantly so. ENT-21,170 and D-D mixture gave relatively poor control, and Nemagon had no apparent effect on the wireworm population. As would be expected from a chlorinated hydrocarbon, SD 4402 exhibited the longest residual action. Am. Cyanamid 18,133 and Diazinon remained toxic longer than parathion. Results with Thimet were inconsistent, but it appears that this material is also longer lasting than parathion. Soil treated with ethylene dibromide was not tested after 36 days, but was not toxic to wireworms after 83 days. Parathion exhibited little residual action after 36 days.

Table 1.—Control of southern potato wireworm in field plots with certain insecticides and residual action of the most effective ones, as shown by laboratory tests of samples of treated soil.

Insecticide	Toxicant per Acre	No. of Surviving Larvae per Sq. Ft.	Per Cent Reduction due to Treatment	Per Cent Mortality[a] of Larvae Put in Treated Soil After	
				36 Days	83 Days
Am. Cyanamid 18133	0.7 lb.	0.2	96	89	33
Thimet	0.94 lb.	0.4	93	6	27
Parathion	1 lb.	0.4	93	0	7
Shell SD 4402	4 lb.	0.4	93	100	80
Ethylene dibromide (40%)	10 gal.	0.4	93	—	0
Diazinon	1 lb.	1.0	82	56	40
ENT-21170	10 lb.	3.2	43	—	—
D-D mixture	19 gal.	3.4	39	—	—
Nemagon	19 gal.	6.0	0	—	—
Untreated	—	5.6	—	—	—
L.S.D. @ 5% level		2.31			
@ 1% level		3.10			

[a] Adjusted for mortality in untreated soil. All mortality records were made 9 weeks after larvae were put in soil.

REFERENCES CITED

Deen, O. T., and F. P. Cuthbert, Jr. 1955. The distribution and relative abundance of wireworms in potato-growing areas of the Southeastern States. Jour. Econ. Ent. **48**(2): 191–3.

Dobrovsky, T. M. 1953. Another wireworm of Irish potatoes. Jour. Econ. Ent. **46**(6): 1115.

Norris, Dale M., Jr. 1957. Failure of soil insecticides to control the southern potato wireworm, *Conoderus falli* Lane. (Abstract) Bull. Ent. Soc. America **3**(3): 40.

Reid, W. J., Jr., and F. P. Cuthbert, Jr. 1956. Resistance of the southern potato wireworm to insecticides. Jour. Econ. Ent. **49**(6): 879–80.

Fumigation Effect of Thiodan against the Green Peach Aphid on Potatoes[1]

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Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) proved to be more effective for control of the green peach aphid (*Myzus persicae* (Sulz.)) on potatoes in eastern Washington when applied from aircraft than with row crop dusters or sprayers. A fumigation effect was suspected, and laboratory tests were made at Union Gap in 1957 to clarify this point. Convergent lady beetles (*Hippodamia convergens* G.-M.) were also used in these tests, since the predation of this and other coccinellid species extended the period of aphid control.

A miscible concentrate containing 2 pounds of Thiodan per gallon was used in all tests. In the first test three ½-gallon glass jars served as fumigation chambers. Six drops of the Thiodan concentrate were placed on a piece of paper towel and suspended in one jar; the same amount of Thiodan was placed in one-half pint of water in the second jar; and the third jar served as an untreated check. Potato leaflets with 25 wingless aphids each were suspended from the lids of the jars, which were then sealed. They were left at room temperature for 1 day before examination. No mortality occurred in the check, but all aphids in the treated jars were dead.

In the second test a Syracuse watch glass with 10 ml. of the Thiodan concentrate was placed in the bottom of a gallon-size jar immediately before the insects were introduced. Four leaflets with 25 aphids and 4 cages containing 2 lady beetle larvae each were placed in the jar. The cages were ¾-inch sections of ¾-inch glass tubing closed at one end with silk gauze and at the other with a cork. The jar was kept sealed except when opened briefly after 15, 30, 45, and 60 minutes to remove leaflets and cages. The room temperature was 77° F. After treatment the insects were kept on a laboratory table until examined. One day later all aphids had survived the exposures of 15 minutes, 18 the 30-minute, and 24 the 45-minute exposure. Ten aphids survived the 60-minute exposure, but all except 1 with wing pads were paralyzed and unable to walk. None of the lady beetle larvae appeared harmed.

The third test was designed to show the length of time that Thiodan would remain effective as a fumigant. One drop of Thiodan concentrate was placed on each of several small glass plates. One plate was immediately sealed in a ½-pint jar with a leaflet bearing 20 aphids. Half the remaining treated plates were placed out of doors in sunlight and the remainder on a table in

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the laboratory. At intervals of 1, 3, 6, 8, and 23 hours a plate from out of doors and one from the laboratory were placed in glass jars with infested leaflets. When examined 1 day later, all aphids were dead in the jar with the freshly treated plate, but no dead aphids were found in any of the other jars, indicating that Thiodan had been lost by an exposure of 1 hour or longer.

In the fourth test, similar to the third, the plates, each treated with one drop of Thiodan concentrate, were exposed to the air in the laboratory only, for 5 seconds and 5, 10, 20, 30, 40, and 60 minutes before being placed in the jars with the insects. Two lady beetle adults and one larva, confined in cages such as were used in the second test, were introduced in each jar in addition to one aphid infested leaflet. The leaflets in the test had an average of 19 aphids per leaflet. On the following day all aphids were dead in the jar with Thiodan that had been exposed for 5 seconds. Based on mortality of aphids in the remaining jars, Thiodan lost half its toxicity in 5 minutes, two-thirds in 10 minutes, nearly all in 20 minutes (2 of 27 aphids dead), and all in 30 minutes or longer. None of these treatments appeared to have any effect on the lady beetles.

The first two tests show that Thiodan is a fumigant in addition to its better-known action as a contact insecticide against aphids. The third and fourth tests show that as a fumigant Thiodan is evolved rapidly when exposed to the air. In thin films on glass its effectiveness as a fumigant appears to have been lost in less than 1 hour. The second and fourth tests indicate that the lady beetles were more tolerant of Thiodan than were the green peach aphids.

The fumigant action of Thiodan appears to account for much of its outstanding effectiveness in large fields treated with aircraft as compared with small plots treated with ground equipment. The survival of the lady beetles further reduces the aphid populations and helps prevent reinfestation. The combination of biological and chemical control avoids the need for a long lasting insecticide with the danger of poisonous residues.

At the conclusion of these tests the writers' attention was called to an article by Finkenbrink (1956), who found that Thiodan was somewhat less volatile than lindane and concluded that the vapor phase of the compound had little toxicity to insects.

Reference Cited

Finkenbrink, W. 1956. Über Thiodan, ein neues synthetisches Insektizid. Nachrichtenbl. deutsch Pflanzenschutzdienst. 8(12): 183–5.

Use of Flashed Opal Glass for Detecting Parasitized Coccids[1]

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The use of light transmitted through the host insect for the purpose of detecting parasitism is not new. During the past 6 years the modification here described has consistently proven superior to simply placing the specimen on a clear glass slide on the microscope stage and adjusting the substage mirror to transmit light up through the subject. Since usually only small numbers of any particular parasite species are received for initial handling in biological control quarantine, and since these are mainly internal parasites whose adults are less than 1 millimeter in length, an improved method of detection without dissection had to be devised.

Experience with flashed opal glass (nationally accepted glazier's term) has conclusively demonstrated that early-instar larvae of such parasites can be seen with certainty through the derm of younger lecanine hosts and in all stages of diaspine hosts. This is rarely possible through clear glass because of refraction and reflection which occur as the rays of light pass through or around the external and internal structures of the host. With flashed opal glass, specimens may be placed either directly on it or on a conventional microscope slide which is then placed on the opal glass. Flashed opal glass diffuses the light and produces a soft uniformity of white illumination which is easy on the eyes and which permits ready visual perception of the immature parasites within the host when viewed through compound or dissecting binocular microscopes.

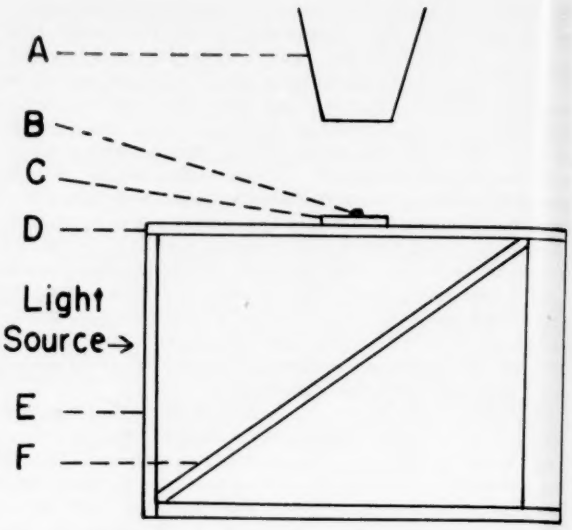

Fig. 1.—Cutaway end view of platform whose dimensions are 6″×4″×15″. The back, ends, and bottom are of wood.

A. Dissecting microscope
B. Subject
C. Microscope slide
D. Flashed opal glass
E. Clear glass
F. Mirror

The light source may be from a substage illuminator or by reflection from the substage mirror shining up through the opal glass. Shown in figure 1 is a platform, the top of which is flashed opal glass and which provides adequate working area for examination of material. It should be mentioned that this device was designed for use with an extension arm-mounted stereoscopic dissecting microscope.

Bonus features are (1) utilization of the ventrally illuminated flashed opal glass as a white background in photography, (2) as a well-lighted work area for gross dissections requiring light to be directly reflected from the specimen, and (3) markedly reduced eye strain.

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Tests with DDT, Lindane and Malathion for Control of the Long-Nosed Cattle Louse *Linognathus vituli* (L.)[1]

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The chlorinated hydrocarbon insecticides have been used extensively in the control of cattle lice. Sprays containing 0.5% of DDT were shown to be effective for 4 weeks against *Haematopinus eurysternus* (Nitz.), and similar results were obtained

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[2] In cooperation with the Animal Disease and Parasite Research Division.

with toxaphene and Strobane (Smith & Richards 1955). In large-scale tests in Wyoming (DeFoliart 1957) 2 to 5 months' control of *Linognathus vituli* (L.), *Solonoptes capillatus* End., *H. eurysternus*, and *Damalinia* (*Bovicola*) *bovis* (L.) was obtained with single applications of chlordane, heptachlor, dieldrin, lindane, DDT, and an organic phosphorus insecticide, malathion. In Oregon and Mississippi rubbing devices treated with chlordane and toxaphene (Hoffman 1954, Neel 1956) also gave a high degree of control of *vituli*, *eurysternus*, and *bovis*. In view of the many reports indicating excellent control of cattle lice with chlorinated hydrocarbon insecticides, the results of recent experiments against *vituli*, the long-nosed cattle louse, at the Agricultural Research Center at Beltsville, Md., seem worth reporting.

On May 16, 1957, the Animal Disease and Parasite Research Division purchased 24 Hereford heifer calves, approximately 3 to 4 months of age, from a cattleman in southwestern Virginia. In line with the general procedure of handling newly received animals, the calves were placed in a disinfected quarantine barn and routinely examined for ectoparasites. About half the animals were lightly infested with *vituli*, and were sprayed with emulsions containing 0.025% of lindane at the rate of 3 to 4 quarts per animal. No lice could be found 1 week after treatment, but a second examination after 18 days revealed light infestations on 6 of the 16 calves still available in the barn. A second spraying was made immediately with 0.035% lindane, and again the 7-day examinations were negative. However, 6 weeks later all but one were again infested with *vituli*, and heavy populations were evident on four of the calves.

At the time of initial treatment it was assumed that the calves had not been treated previously, but it was later learned that they had received two treatments of 0.075% lindane emulsion applied by a spray-dip machine. The first treatment had been made late in March and the second about 2 weeks before the animals were shipped to Beltsville. The fact that these two treatments by the owner and two additional treatments at Beltsville failed to eradicate the lice suggested the possibility of resistance. Additional tests were therefore made to determine if resistance had developed and to compare the effectiveness of several insecticides.

CALF-SPRAYING TESTS.—Twenty of the original 24 calves were available for these tests. Pretreatment counts of lice were made and infestations in the heaviest area on the animal were classed as follows:

Heavy—5 or more lice per square inch.

Medium—less than 5 per square inch, but 5 or more per 9 square inches.

Light—less than 5 per 9 square inches.

Eighteen of the calves were then divided into three groups and placed in different field pens for treatment. Two lightly infested calves were retained as controls. Emulsions containing 0.075% of lindane, 0.5% of DDT, and 0.5% of malathion were applied to the respective groups at the rate of 4 to 5 quarts per animal. The sprayer was of a portable pneumatic type and was operated at a pressure of 40 to 45 p.s.i.

Lindane apparently gave complete kill of all motile lice, and none were detected at the 7-day examination. After 14 days light infestations of nymphs and adults were observed. At 30 days some of the calves showed lice populations as high as before treatment.

DDT gave excellent immediate control of lice, but 4 of the 6 calves had light infestations of nymphs after 7 days. After 30 days the infestations were about the same as before treatment.

The malathion treatments were completely effective throughout the 30-day period. There were no apparent changes in the lice populations on the two calves used as controls.

LABORATORY TESTS.—Since these tests indicated some resistance, especially to DDT, laboratory tests were made to compare the susceptibility of lice from the Beltsville and Virginia cattle. Preliminary trials were conducted to determine how long adult cattle lice would survive off the host. The lice were placed on filter paper in beakers and held at room temperature of 75°–80° F. and relative humidity of 60–80%. At least 80% of the lice survived 24 hours, indicating that tests could be conducted satisfactorily by the techniques used with human body lice (King 1954).

The two strains of lice were compared by continuous exposures on No. 3 Whatman filter-paper disks treated with acetone solutions of DDT at 5 and 25 mg. per square foot, lindane at 5 mg. and malathion at 25 mg. The treated disks were allowed to dry 4 hours before testing.

The Virginia lice were selected from two calves that had received the following treatments of lindane: late March, 0.075%; late April, 0.075%; May 12, 0.025%; May 30, 0.035%; and August 6, 0.075%. The Beltsville lice were taken from two Holstein heifers that, according to dairy records, had never been treated with an insecticide.

Two tests were conducted. In the first test 5 mg. of DDT was compared with 5 mg. of lindane and 25 mg. of malathion. In the second test the dosage of DDT was increased to 25 mg., but the lindane and malathion dosages were not changed. Adult lice were picked from the donor animals, returned to the laboratory and promptly placed in test. The tests with the Beltsville lice were run in quadruplicate and those with the Virginia lice in triplicate. Each beaker contained 10 lice, the ratio of males to females was equal in most beakers and never greater than 6 to 4 in favor of either sex. Control beakers, two for each strain of lice, were included in each test. The numbers of dead or moribund lice were recorded hourly for 8 hours and again after 17 and 24 hours.

Provisional regression lines were fitted to probit mortality data on logarithmic normal paper and the median lethal time required to kill 50% of the lice was read directly. The results of the tests are given below:

	MG./SQ. FT.	LT-50 (HOURS)	
		Beltsville	Virginia
DDT	5	8.4	15.9
	25	4.9	7.9
Lindane	5	2.7	3.5
Malathion	25	4.8	3.2

The Beltsville lice appeared to be about twice as susceptible to DDT as the Virginia lice. On the 5-mg. deposits some of the Beltsville lice were seriously affected after 1 hour, whereas the Virginia lice were not visibly affected until 7 hours. After 10 hours 70% of the Beltsville and 16% of the Virginia lice were moribund or dead, and 17-hour counts indicated 90 and 54% mortality, respectively. On the 25-mg. deposits some of the Beltsville lice were knocked down in 30 minutes, whereas knockdown of the Virginia lice was not observed until after 3 hours. At 8 hours the respective mortalities were 80 and 50%, and at 17 hours 100 and 90%. The greatest mortality in the controls was 10%.

Although the failure of lindane to control lice on the Virginia calves prompted these experiments, the LT-50s indicated little resistance to lindane. After 3 hours' exposure to 5 mg., 30% were dead or moribund, as compared with 50% of the Beltsville lice. After 6 hours the percentages were nearly the same for the two strains.

Both strains of lice were highly susceptible to malathion, the Virginia lice being slightly more so than the Beltsville strain. This difference may have been due to the fact that fewer Virginia lice were used in the test or to normal experimental variation.

Although this is believed to be the first attempt to show resistance in cattle lice, reports from other sections of the United States have indicated increased difficulty in controlling these pests. Although the results of the current tests do not prove

their resistance to DDT, the difference in knockdown times does indicate a marked difference in susceptibility, and this is supported by the twofold difference in the LT-50.

REFERENCES CITED

DeFoliart, G. R. 1957. Lice control on northern range herds with residual sprays. Jour. Econ. Ent. **50**(5): 618–21.

Hoffman, Robert A. 1954. Self-treatment rubbing devices for louse control on cattle. Jour. Econ. Ent. **47**(4): 701.

King, W. V. 1954. Chemicals evaluated as insecticides and repellents at Orlando, Fla. U. S. Dept. Agric. Handbook **69**, 397 pp.

Neel, William W. 1956. Tests with self-treating devices for the control of lice on cattle in Mississippi. Jour. Econ. Ent. **49**(1): 138–9.

Smith, Charles L., and Rowland Richards. 1955. Evaluations of some new insecticides against lice on livestock and poultry. Jour. Econ. Ent. **48**(5): 566–8.

Biological Control[1]

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The basis of the biological method of population control, as amply demonstrated in agriculture and in wildlife management, is Nicholson's (1933) law that the "action of the controlling factor must be governed by the density of the population controlled." The amount (density) of food governs the self-regulating action of subsisting populations. However, in the production and conservation of food for human consumption it is essential that its abundance not be subject to control by the various animals which can feed upon it. Agriculture circumvents such control by suppressing the unwanted populations either by applications of lethal chemicals or by manipulating the environment so that such populations serve as food for efficient natural enemies.

The self-regulation of certain predator-prey (parasite-host) associations results in the population densities of such associations being maintained well within their means of subsistence. Fortunately for plant life this is a common type of biotic regulation, perhaps the most common as far as animals are concerned.

The existence of healthy populations of plant-feeding animals has long been known to be dependent on predation. Wildlife management is predicated on the fact that predators under natural conditions may preclude the depletion of plant life and the consequent erosion of top soil. They thus indirectly ensure the steady flow of life-filled streams. As stated by Leopold (1939) only wolves and lions can ensure the forest against destruction by deer and ensure the deer against self-destruction.

The inherent fecundity of plant-feeding animals greatly exceeds any possible food supply. Since man is also dependent upon plants for food, he has found it necessary to manipulate the natural enemies of other plant feeders as a means of conservation, the objective being to conserve plant material either directly as plants or indirectly in the form of plant-feeding animals.

Consequently, we see on one hand the agriculturalist utilizing "biological control" to reduce to noneconomic densities the animal populations destructive to plants and on the other hand the wildlife ecologist utilizing "defensive control" to protect the carrying capacity of forest, field, and stream so that the yield of animal life is sustained at its maximum.

These "controls" consist of the deliberate extension or intensification of natural processes (natural enemy manipulation) which tend to limit reproduction at more or less permanent densities (Allen 1942). Wildlife management largely consists of so manipulating the environment as to change it more or less permanently either in favor or in disfavor of the animal species.

The increase of natural predation is encouraged in order to prevent an animal population from exceeding the optimum carrying capacity of its habitat. Hunting and trapping, when limited to surplus animals, is a density-dependent phenomenon and thus simulates natural regulation.

Biological control utilizes the processes involved in the natural regulation of populations but as a practice is limited to reducing the cost of controlling the pests of agriculture. To paraphrase the words of Allee & Schmidt (1937) biotic control, despite obvious difficulties, has become a standard procedure in pest control by which man attempts to restore a balance of nature which has been destroyed either by himself or by some natural catastrophe.

An insect or a plant that has attained pest status because of the reduced efficiency of its natural enemies resulting from cultural practices (density independent factors) is biotically controlled if by some modification of such cultural practices balance is re-established at reduced population levels. Biotic control, therefore, may involve the modification of a density-limiting host-parasite interaction by abiotic factors (Nicholson 1933).

Early in its development the main objective of biological control, as stated by L. O. Howard in his "Outlook for the Biological Method of Fighting Insects" (1916 Yearbook of the U. S. Department of Agriculture), was merely to reproduce in the region where an insect is a pest the entire parasite complex to which that insect in its native habitat is subject. The outlook as then seen by Howard was for ". . . a very considerable development of this method . . . ." Fourteen years later, however, in his "History of Applied Entomology," Howard felt constrained to write, "I do not waver in my unfailing belief in the basic value of the principle of biological control, but my outlook becomes more or less confused when I consider the complications." The "complications" considered by Howard were, in part at least, the highly involved aspects of population dynamics as delineated by Smith (1929).

This discussion of biological control may be said to be presaged by the complaint of Howard (1930) that the simple importation in numbers of the parasitized stages of the pest insect ". . . is now referred to condescendingly by recent workers as 'the old method' . . . ." The simple importation of an insect population, however, is, in the terms of population dynamics, manipulating biotic balance.

Each species of animal exists as a balanced population. Balance is, in fact, the *sine qua non* for animal existence. Animal populations, therefore, oscillate around a mean or steady density, this density being subject to change as are the amplitudes of the oscillations. Populations expand and contract as the pressure of the environmental resistance decreases and increases. The level of the steady density, however, may be either raised or lowered by cultural practices, by the periodic application of chemicals, or by the addition of natural enemies, the population dynamics involved being the same in each case.

The biotic mechanism (balance of nature) may involve all associated species. It is a reciprocal action involving either a population's food supply or its natural enemies. Fortunately for agriculture food-induced balance between a plant-feeder and its natural enemy precludes a plant-depletion-induced balance. Preclusion of the plant-depletion-induced balance is the *raison d'etre* of biological control.

To the agriculturalist biological control is a means of changing the density of a pest population in but one direction—from an economic high to an economic low. In relatively few instances, however, can a reduction from an economic to a completely noneconomic status be expected. *As a practice* biotic control is

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either discontinuous, ending when the balance is re-established (natural enemy regulation occurring at a low level of density) or continuous, the pest population then being held at a non-injurious density either by certain cultural practices that augment the effect of the natural enemy population or by periodic colonization of natural enemies.

Biological control, in its *employment* of biotic and abiotic factors, is an artificial procedure. However, an adjusted balance resulting from the addition of an exotic enemy is natural if it operates thereafter unassisted by man.

Because of established usage, "biological control" denotes not only pest control through the manipulation of natural enemies, it denotes also the balance attained by any population whose level of density results from the action of natural enemies.

The practice of biological control connotes intensive and extensive investigations in the field of population dynamics in order to evaluate the economic effects of augmented biotic resistance on pest populations and of applications of insecticides on natural enemy populations.

References Cited

Allee, W. C., and K. P. Schmidt. 1937. Ecological animal geography. John Wiley & Sons, Inc., N. Y. 597 pp.

Allen, P. F. 1942. Defensive control of rodents and rabbits. Jour. Wildlife Management **6:** 122–32.

Howard, L. O. 1930. A history of applied entomology. Smithsonian Misc. Coll. **84,** Washington, D. C. 564 pp.

Leopold, Aldo. 1939. A biotic view of the land. Jour. Forest. **37:** 727–30.

Nicholson, A. J. 1933. The balance of animal populations. Jour. Animal Ecol. (Suppl.) **2:** 132–78.

Smith, H. S. 1929. Multiple parasitism: its relation to the biological control of insect pests. Bull. Ent. Res. **20:** 141–9.

Chemical Control of Army Cutworms in Sugar Beets[1]

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The army cutworm, *Chorizagrotis auxiliaris* (Grote), has caused extensive injury to small grains, alfalfa, and sweetclover in Kansas for many years. Damage in some years has been widespread. Even when total damage is comparatively small on a state-wide basis, this cutworm may be highly important to an individual grower whose crops are destroyed. Burkhardt (1954) reported on control of this species in alfalfa in northeastern Kansas. Pfadt (1955) reported on control in alfalfa which often is infested in the spring when feeding of overwintered larvae on young shoots may prevent their emergence above ground litter.

Early spring feeding by army cutworms severely injured seedling sugar beets during the spring of 1958 in southwestern Kansas, west of Garden City. The infestation was concentrated in one corner of a 35-acre field.

Methods and Materials.—The infested area was divided into four blocks of eight plots each. Each plot was 6 rows wide and 25 feet long. Seven treatments and one check were assigned at random to each block. Treated plots were separated by two-row buffer zones left untreated throughout the experiment.

Populations were determined by counting larvae in four randomly selected 1-square-foot samples in each plot. Effectiveness of treatments was evaluated by comparing difference in numbers of live cutworms between treated and untreated plots 7 days after treatment. The cutworms were judged to be predominantly fourth and fifth instar larvae at treatment.

Insecticides used and rates of application are given in table 1. Insecticides were applied to the soil surface and to plants with a portable compressed-air hand sprayer with an 18-inch boom, having three cone-type nozzles. The emulsions were applied at 17 gallons per acre with a constant pressure of 20 p.s.i. Application was made during the late afternoon at approximately 75° F. temperature.

Table 1.—Insecticidal control of army cutworms in sugar beets near Garden City, Kansas, May, 1958.

Treatment[a]	Pounds Actual Toxicant per Acre	Mean No. Live Cutworms per Sq. Ft. (7 Days)	Per Cent Control After 7 Days
Endrin, 19.5%	0.2	0.25	94
TDE, 25.0%	2.0	0.75	83
Dieldrin, 18.6%	0.375	1.00	78
Heptachlor, 23.4%	0.5	2.25	50
Aldrin, 23.8%	0.5	2.50	44
Toxaphene, 60.5%	2.0	3.00	33
Thiodan, 24.0%	1.0	3.00	33
Check	—	4.50	—
L.S.D. at 5% level		1.98	
at 1% level		2.69	

[a] Materials were all emulsions supplied as follows: endrin, dieldrin, and aldrin by the Shell Chemical Corporation, New York, N. Y.; TDE by the Rohm & Haas Co., Philadelphia, Pa.; heptachlor by the Velsicol Corporation, Chicago, Ill.; toxaphene by the Hercules Powder Co., Wilmington, Del.; and Thiodan by the Niagara Chemical Division, Middleport, N. Y.

Results.—The relative effectiveness of the insecticides tested is summarized in table 1. In this test, endrin resulted in 94% control; TDE, 83% control; and dieldrin, 78% control. Control in the remaining treatments ranged from 33 to 50%.

Because of the severe reduction in beet stand in the infested area of the field, the grower subsequently worked the soil to plant another crop, so a 14-day mortality count could not be taken.

Discussion.—Although each treatment reduced the infestation below that of the untreated check, only endrin, TDE, and dieldrin gave satisfactory control under conditions of this test. Analysis of variance showed all three treatments significantly better than others at the 5% level. Heptachlor, aldrin, toxaphene, and Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) failed to give practical control at the end of 7 days. Although heptachlor and aldrin were inferior to the three best materials, the numbers of live cutworms were significantly less in these plots than in the untreated check.

This test corroborates the results obtained in Wyoming by Pfadt (1955) who found that endrin was the most toxic material, followed by dieldrin, heptachlor, toxaphene, and aldrin, in that order, for control of army cutworm in alfalfa. Burkhardt (1954) also found endrin to be the most effective insecticide for control of army cutworms in alfalfa in Kansas. However, the poor control obtained with heptachlor and aldrin in this test does not confirm results by Burkhardt who found those two materials fairly effective in controlling army cutworms in alfalfa.

References Cited

Burkhardt, C. C. 1954. Control of army cutworms. Jour. Econ. Ent. **47**(6): 1156–7.

Pfadt, R. E. 1955. Control of army cutworms in alfalfa. Jour. Econ. Ent. **48**(2): 227.

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Tubulifera Thrips Damage Lettuce[1]

J. G. WATTS[2]

A species of thrips of the suborder Tubulifera discovered on March 10, 1959, doing serious damage to a field of young lettuce at Mesquite, New Mexico, has been identified as *Haplothrips clarisetis* Priesner.[3] A competent observer reports having seen them on lettuce in the same area in the fall of 1958. This is an African species (Egypt and South Africa) apparently introduced into this country only recently. Cabbage and lemon have been listed as hosts in South Africa (1937) without indication of economic damage. The damaged lettuce was in the 4- to 8-leaf stage and there were up to 3 to 4 dozen adult females (no males were found) on some plants. Typical thrips injury was observed on the under surface of the leaves, and an estimated 50% or more of the plants had been killed. A few larvae were found, as were some small eggs, which could easily be those of this species. A tendency toward gregarity was observed in that some plants had numerous thrips, while adjacent or nearby plants had only a few or none. A considerably lower level of infestation was found in other fields at Las Cruces, Mesilla, and Dona Ana, New Mexico. Light infestations were also found on onions at Mesilla, Mesilla Park, Las Cruces, and Fairacres, New Mexico.

Dr. H. W. Ewart (1959), Citrus Experiment Station, Riverside, California, reported the occurrence and apparent recent introduction of *H. clarisetis* into southern California but observed no economic damage associated with it.

H. clarisetis is very dark brown to jet black except that the wings are silvery or whitish. This is in contrast to the yellow to medium brown color of the *Frankliniella* spp. and *Thrips* spp. more frequently found damaging broadleaf cultivated crops. It is about one-third larger than the onion thrips (*Thrips tabaci* Lind) or the western flower thrips (*F. occidentalis* (Perg.)).

The most striking fact regarding *H. clarisetis*, in contrast to other species of thrips that are of economic importance on cultivated crops in this country, is that it belongs to the suborder Tubulifera. The currently recognized major pest species of thrips in the United States belong to the suborder Terebrantia. In other parts of the world there are pest species which belong to the Tubulifera. The future economic importance of *H. clarisetis* in the United States cannot be predicted, but it has demonstrated the capability of doing serious damage to lettuce.

REFERENCES CITED

Ewart, W. H. 1959. A thrips *Haplothrips clarisetis* Priesner. Cooperative Econ. Insect Rept. 9(12): 193.

Jacot-Guillarmod, C. F. 1937. Ten new species of Thysanoptera and a catalogue of the known South African forms. Publications of the University of Pretoria, Series II: Natural Science No. 3, pp. 62.

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[3] Identified independently by Miss Kellie O'Neill, Entomology Research Division, U. S. Department of Agriculture, and L. J. Stannard, Jr., Illinois Natural History Survey.

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Entomological Society of America
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OBITUARIES

Charles Lewis Fluke
1891–1959

The death of Emeritus Professor Charles Lewis Fluke on February 11, 1959, brought peace to a man weakened and weary by several years of illness. The University of Wisconsin lost a distinguished teacher and biologist. His memory will be cherished by all of those who knew him for he was a man of kindly spirit, and one who gave his talents to unselfish service to the citizens of Wisconsin.

Charles Fluke was born on a farm in Grand Junction, Colorado, on August 7, 1891. He showed a keen interest in insects as a boy, and majored in the study of insects while an undergraduate at Colorado Agricultural College. Following his graduation with a B.S. degree in 1916, he joined the staff of the Department of Entomology at the University of Wisconsin to continue his graduate study on an assistantship appointment. He obtained his M.S. degree in 1918, his Ph.D. in 1928, and reached the rank of professor in 1928. During the war years of 1942–46, he served as chairman of the Department of Entomology at Wisconsin. He was raised to Emeritus rank on July 1, 1958, culminating 42 years of continuous service in the University.

Professor Fluke showed wide versatility in his professional career, making significant contributions in teaching, systematics and economic entomology. The effectiveness of his teaching of undergraduate students brought him recognition as one of the outstanding instructors in the College of Agriculture. He carried a heavy undergraduate and graduate teaching repsonsibility in his Department for many years. During that period his unfailing enthusiasm and cooperation encouraged many students towards higher endeavor in entomology.

Summer months were devoted in large part to Agricultural Experiment Station projects on the biology and control of insects harmful to the production of fruits and vegetables. He published 32 technical articles on this research, and in addition wrote many articles of popular nature for the farmer. He was much in demand for speaking engagements at meetings of farmers, fruit growers, and garden clubs, as he had great ability to share his broad knowledge in practical terms. In recognition and appreciation of his contributions to the welfare of agriculture, he was honored by the Wisconsin Horticultural Society in 1949.

Probably his most absorbing interest was research on the biology and systematics of the Syrphidae. These dipterous insects, so important as predators of aphids and other insect pests, were the subject of his doctorate research. During his many years of study on the North and South American Syrphid fauna, he became a world authority on the systematics of the group. His collected writings including catalogs, monographs and numerous technical articles describing new species, are equal in size to two large volumes. Many of his publications have appeared in Jour. Econ. Ent., Ent. News, Jour. Kansas Ent., Annals Ent. Soc. America, Wis. Academy of Sci., Arts & Letters, Revista Brasileira de Entomologica and others. His two most recent publications are entitled "A Study of the Male Genitalia of Melanostomini (Diptera-Syrphidae)" in 1957 and the "Catalogue of the Family Syrphidae in the Neotropical Region (Diptera) Part I" in 1956. His reputation in the systematics of these insects led to grants by Sigma Xi and the American Museum of Natural History for support of his basic research.

Charles Lewis Fluke
1891–1959

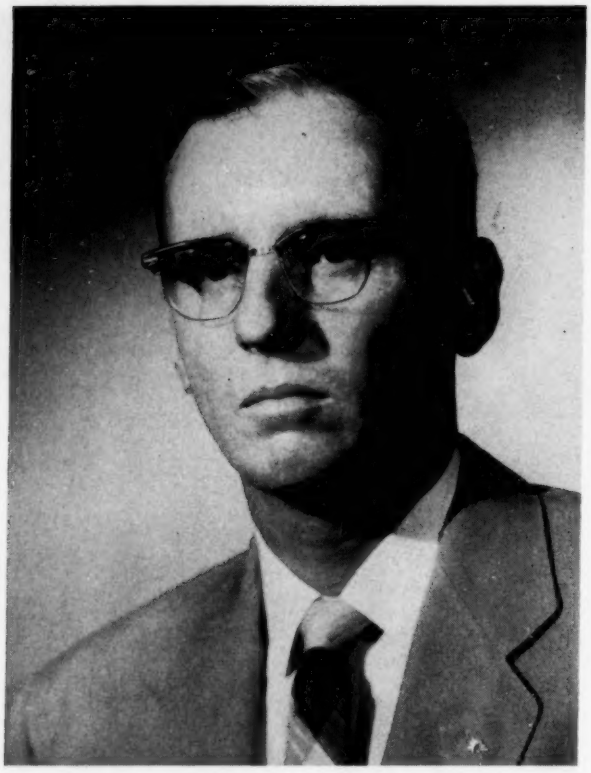
In accordance with his wishes, Dr. Fluke left his literature and the bulk of his Syrphid collection to the Entomology Department of the University of Wisconsin. The Entomology Department is establishing a C. L. Fluke Entomological Library with the Fluke literature as a start. Separates or entomological books to be added to this library in his name as a memorial to him would be welcomed.

Professor Fluke was active in the Wisconsin Academy of Science, Arts and Letters, and served as President of the Academy in 1954. He did much in building up the membership of the Academy, and was deeply aware of the importance of the Junior Academy in stimulating young scientists. He was a member of Sigma Xi and Phi Sigma.

The personal affairs of his students and the social activities of his department and the University occupied a good portion of Professor Fluke's spare time. He was keenly interested in his University colleagues, and the Faculty Bowling League in which he was a prominent figure and loyal supporter is an example of this continued interest in his associates. He was also very active and influential in his church affiliations.

Professor Fluke is survived by his wife, Myrtle; a son, Barnaby, Milwaukee; two daughters, Mrs. Clarence Chrest, Kalamazoo, Michigan and Mrs. Richard Ortmeyer, Dallas, Texas; and seven grandchildren.

T. C. Allen



Freeman M. Fuller, Jr.
1924–1959

Dr. Freeman M. Fuller, Jr., entomologist for the Texas Agricultural Extension Service for the past 6 years, died at his home in Bryan, Texas, March 12, 1959. He was 34 years of age.

Dr. Fuller was born near Selma, Alabama, and received two degrees from Alabama Polytechnic Institute, a B.S. in agricultural science in 1947 and a master's in entomology in 1950. He was awarded a Ph.D. degree in 1954 in entomology at Texas A. & M. College. Before coming to Texas A. & M. College in 1951, he was employed by a commercial insect control service in Alabama.

As extension entomologist, Dr. Fuller was in charge of the entomology subject matter program for the Extension Service in Texas. He worked in all fields of entomology and was the author or co-author of many publications dealing with insects and their control. As a member of the State 7-step cotton program team, Dr. Fuller played a leading role in conducting the insect control part of the program and his efforts gained for him state and national recognition. He gained further stature as an entomologist from his efforts in fighting the imported fire ant.

At Alabama Polytechnic Institute, Dr. Fuller was listed in Who's Who Among Students in American Colleges and Universities; was a member of Spades; Alpha Zeta; Delta Kappa; Alpha Gamma Rho; Gamma Delta Gamma: editor of Alabama Farmer, and A Squirer. He was listed in the American Men of Science.

Dr. Fuller was a member of the Methodist Church; Entomological Society of America; many learned and professional groups and a 32nd Degree Mason, Knights Templar and Shriner. He served in the U. S. Army from 1943 until 1945 and was wounded in action in Germany. His death was from causes related to his battle wound.

Dr. Fuller is survived by his wife, Mrs. Marie Marschall Fuller and an infant daughter, Cynthia Carolyn, and by his parents Mr. and Mrs. F. M. Fuller, Sr., Perryville, Alabama and a brother, Tom, of Greenville, South Carolina.

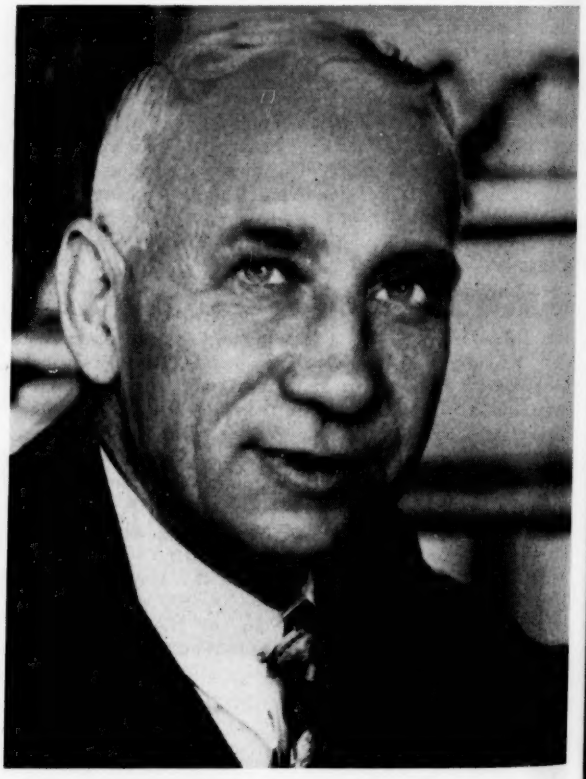
J. W. POTTS

Harley Frost Wilson
1883–1959

Harley Frost Wilson, Professor of Entomology 1915–48, died at his home in La Canada, California, February 8, 1959. Professor Wilson was born at Del Norte, Colorado, April 14, 1883, received his B.S. degree at Colorado State College in 1907 where he majored in Entomology and Horticulture. He worked for the State of Illinois Nursery Inspection Service and did graduate work at the University of Illinois 1907–08; served as special agent in the U. S. Bureau of Entomology at Washington, D. C. 1908–1910; and was research assistant in Entomology at the Oregon State College 1910–13. He received the M.S. degree at Corvallis in 1913 and was appointed Professor of Entomology and Entomologist of the Experiment Station in Oregon at that time.

Professor Wilson joined the faculty of the University of Wisconsin in 1915 to head the Department of Economic Entomology in the College of Agriculture where he served with distinction as its Chairman until 1942. He retired from the University in 1948, at which time he moved to La Canada to serve as Research Director for Pickett & Eckel, Inc., manufacturers of metal slide rules. Mr. R. C. Pickett, President of the firm, received his B.S. in 1915 and M.S. in 1921 in Entomology at the University of Wisconsin under the direction of Professor Wilson.

Harley Wilson was "Prof" to his associates and his students. He was a thorough teacher who expected full preparation and



Harley Frost Wilson
1883–1959

maximum effort, yet he was an understanding counselor to all. Twenty students earned their doctorate degrees under his direction. Many of these are now leaders in various phases of entomology because of his understanding supervision and personal encouragement during their graduate years. In 1927 Professor Wilson edited the first issue of the "Wisconsin Hexapod," a Department of Entomology newsletter which now has become a Wisconsin tradition through its annual distribution to former and present faculty and staff members.

In research he had many interests contributing to the taxonomy of Aphidae and the biology and economic control of major insect pests of Wisconsin. Two major papers on the taxonomy of aphids were published by Professor Wilson. One paper appeared in 1911 entitled "Two new genera and seven new species of the family Aphididae," and the other in 1918 which contains "A species list of the Aphididae of the world and their recorded food plants." As his staff increased, he concentrated principally on pea aphids, household insects, and beekeeping. Important contributions to his credit include the electrostatic effects of rotenone diluents, the accumulation of DDT in animal fats, attractants to improve control measures against clothes moths and carpet beetles, and in beekeeping his research on wintering, nutrition, diseases, and effects of yeast on honey storage stand out.

The Dr. C. C. Miller Memorial Beekeeping Library accepted by the University of Wisconsin August 8, 1922, will be a permanent monument to the vision and energies of Professor Wilson. In 1929 he enlisted a donation by the late Siguard L. Odegaard to purchase the world's largest private beekeeping collection from Lt. Col. H. J. O. Walker of Budleigh Satterton, Devon, England. Without a doubt, the Miller Library contains the largest collection of beekeeping literature to be found any place in the world, and in 1930 a conservative estimate of its value was placed at $12,000. He played a guiding part in the foundation of the American Honey Institute that has functioned successfully for more than 30 years. He initiated and edited Wisconsin Beekeeping 1924–31, when it was absorbed by the Wisconsin Horticulture magazine. He held many offices as an advisor to the Alpha Gamma Rho fraternity, including Grand President, Chapter Inspector, and served on its Board of Endowment Funds.

Harley Wilson was a scientist, teacher, and a man with great energy who found time to counsel others and to enjoy the outdoor sports of hunting and fishing. His deep personal interest in his students and his eagerness to help them, even in concrete economic ways, will be remembered by them with lasting gratitude and affection. He is survived by his wife, Clara; and two sons, Robert and John, who reside at La Canada, California.

T. C. ALLEN

Clell Burns Wisecup
1902–1959

On February 1, 1959 a well known and outstanding entomologist died in Manhattan, Kansas, at the age of 57 years, after a long illness.

Bennie, as we all referred to him, was born July 31, 1902 at Pulaski, Iowa. He was graduated from Kansas State College with the degree of Bachelor of Science in 1926 and completed graduate work for the M.S. degree at Kansas State in 1928.

His professional career began with employment by the U. S. Department of Agriculture, Bureau of Entomology, later designated as the Bureau of Entomology and Plant Quarantine. In the summer of 1928 he received an appointment at the Truck Crop and Garden Insects Laboratory of the U. S. Department of

Clell Burns Wisecup
1902–1959

Agriculture at Sanford, Florida. This pioneer work on control techniques made an invaluable contribution for the control of pests of celery, cabbage, and other truck crops. In 1942 the Office of Scientific Research and Development made a contract with the USDA to conduct investigations at the Orlando, Florida, laboratory on the control of insects and other arthropods of importance to the Armed Forces in World War II. Mr. Wisecup was selected to serve on the staff of this laboratory. His research was superior on special programs to develop more effective methods for controlling mosquitoes, flies, and other arthropods which attack and transmit diseases to man. In this capacity he made outstanding contributions to the war effort and to the public during and since the war. In 1946 he was appointed Entomologist on the staff of the Engineer at Headquarters, Seventh Service Command, Omaha, Nebraska. In 1947 he was transferred and promoted to a similar position of Entomologist at Headquarters, Second United States Army, Fort George G. Meade, Maryland. During service with the Department of the Army, 1946 until retirement 30 June 1954 because of a serious and incapacitating illness, Mr. Wisecup played a key role in the development of policies and procedures for post engineers in the supervision and execution of insect and rodent control measures at Army installations. These contributions included standards for selection and training of personnel; standardization and supply of equipment and pesticides; protection and transportation of toxic materials; aircraft dispersal of insecticides; and the application of pesticides by post engineers on property near military installations. In recognition of this meritorious service the Secretary of the Army presented him with the Exceptional Civilian Service Award.

Mr. Wisecup's membership in scientific societies included the American Association for the Advancement of Science, the

American Association of Economic Entomologists, the Entomological Society of America, the Florida Entomological Society, and the American Mosquito Control Association.

He is author or co-author of more than 40 papers relating to research, operations, and administration for the control of insects and other pests.

Surviving Mr. Wisecup are his wife Jeanie; daughter Miss Emma Jean Wisecup; a son, Dr. W. G. Wisecup, Base Veterinarian, Holloman Air Force Base, New Mexico; and grandchildren Robby and Kay.

Bennie was held in highest esteem for his good judgment and qualities of leadership. He set us a high standard for hard work, exemplary personal habits, and complete devotion to the science of entomology.

A. W. LINDQUIST
W. D. REED

Notes on Control of Mange Mites in Laboratory White Mice with Malathion[1]

JOSEPH E. WEBB, JR.[2] and EDWIN L. SHEPHERD[3]

During April and May 1958, a severe outbreak of mange occurred among the some 400 mice in the USAREUR Medical Laboratory white mouse colony. By the time the Entomology Department was called upon to assist in control of the mites, there had been almost 50% mortality of the mice. Washings of several of the mice yielded many specimens[4] of *Myocoptes musculinus* (Koch), and scrapings from the mange scabs yielded numerous *Myobia musculi* (Schrank).

Control measures noted as recommended in the literature include use of such acaricides as sulfur, Aramite®, dithocyanodiethyl ether and lindane (Baker *et al.*). Davis (1957) reported using malathion as a dip and in litter treatment to control *M. musculinus*.

In this report, malathion was compared with lindane for use in treating the bedding to control the above-named two species of mites on the infested laboratory colony.

MATERIALS AND METHODS.—A series of eight 2-gallon glass animal jars were each supplied with 85 gms. of bedding (wood shavings). Four of these jars were treated by thorougly stirring into the shavings 75% lindane powder at rates ranging from ½% to 6% by weight of actual lindane. The other four jars were treated by mixing into the bedding 25% malathion powder at rates ranging from ½% to 6% weight of actual malathion. Then a series of 30 adult mice were selected at random from the infested colony. Four of these were washed separately with detergent in a jar of warm water, and about 25 to 40 mites recovered from the washings from each animal. Scrapings from mange scabs of two other mice of the group yielded 30 specimens of *Myobia musculi*. Three of the remaining mice were then placed in each of the eight jars with treated bedding. One additional jar was treated at the rate of 6% by actual weight of malathion in the litter and used for a female with a litter of six 1-day-old mice. All mice were fed, watered and checked daily for apparent illness.

RESULTS.—At the end of 1 week the mice in the lindane-treated jars were all either sick or dead, while all mice (including the suckling young in the malathion treated jars were normal, even at the highest dosage rate. At this time the adult mice from the malathion-treated jars were killed and examined for the presence of mites. None were found on any of the animals either by scraping mange scabs or by washing in warm water and detergent and examining filtered residue under the dissecting microscope.

COMMENTS ON PRACTICAL APPLICATION.—Laboratory Animal-House personnel began on June 1, 1958, treating all bedding for the entire mouse colony at the rate of 1 gm. of 25% malathion powder per jar of about 85 gms. of bedding; *e.g.*, about 0.3% actual malathion by weight. Treatment was repeated approximately once weekly when the bedding was changed. The mange condition cleared up completely after about 6 weeks continuous use of treated bedding. Examination of several mice from the colony during the ensuing weeks revealed no mites present.

In September, after about 15 weeks' use, the malathion treatment of the colony was discontinued. In November, the mange condition began to reappear. It is not known whether this was due to reinfestation from outside mice being brought into the colony or from a residual of the old infestation within the building. In any event, the use of malathion at the previous dosage rate in bedding was resumed but this time by using 57% emulsion concentrate in lieu of the powder. This was accomplished by sprinkling over the some 85 gms. of bedding in each jar a mixture of ½ ml. of the 57% concentrate diluted with 5 ml. of water. The same apparent results were obtained as previously, and this procedure has been standardized for permanent control of the two species of mange mites. This dosage of malathion emulsion (or powder) applied regularly in the bedding has been arrived at as a practical and effective means of control of mites and probably of other arthropod infestations of white mice at the USAREUR Medical Laboratory, Landstuhl, Germany.

REFERENCES CITED

Baker, E. W., T. M. Evans, D. J. Gould, W. B. Hull, and H. L. Keegan. 1956. A Manual of parasitic mites. National Pest Control Association, 250 West Jersey Street, Elizabeth, New Jersey.

Davis, Robert. 1957. Control of the myocoptic mange mite, *myocoptes musculinus* (Koch), on laboratory mice. Jour. Econ. Ent. **50:** 695.

[1] Contribution of the Entomology Department of the U. S. Army, Europe Medical Laboratory at Landstuhl, Germany. Assistance of medical Technicians, Raymond C. Lints and John B. Barton is gratefully acknowledged. Accepted for publication April 1, 1959.

[2] Lt. Colonel, U. S. Army, MSC, Chief, Department of Entomology, USAREUR Medical Laboratory, Germany.

[3] Sergeant, First Class, U. S. Army, Chief, Preventive Medicine Technician, USAREUR Medical Laboratory, Germany.

[4] Determinations made by Major Hugh L. Keegan, U. S. Army, Department of Preventive Medicine, AMSS, Fort Sam Houston, Texas.